Gratis

PECTIN -Its Manufacture, Properties and Uses
By William M. E. ELWELL

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PREFACE

The main purposes of this bulletin are to review the knowledge of pectin, recite its economic aspects and stimulate interest in it, for the advantage of the State of Washington. The material embodied covers the various aspects of pectin from its uses in the home to purely physical and chemical considerations. It presents information of interest to the public, the manufacturer, and the chemist. The literature is reviewed and is supplemented with knowledge of pectin developed at the University of Washington during the past three years. These studies were made possible by the Federal Grant, W. P. A. No. 65-93-1336 for which acknowledgement is gratefully made.

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INTRODUCTION

Whereas the fruit industry of the State of Washington is one of our mos important sources of wealth, it has not been promoted and developed to stable, maximum capacity. Owing to the rapidly perishable nature of som fruits, but more especially owing to substantial lack of planned marketin and varied processing and active publicizing for sales, this industry has no advanced in the same manner and to the same degree as it has in certain o our sister states. It is obvious that the acreage under cultivation could b greatly increased provided an ample market were made available for absorp tion of the crop. Also it is reasonable to conclude that increased absorptio of fruit can be promoted if a greater variety of products is developed, espe cially if the quality of these and the attractiveness of containers are perfecte and highly advertised. The following outline recites the forms in which th raw material is now absorbed by markets:

1. Fresh fruit, mostly for local markets.
2. Cold storage fruits for the continuous market.
3. Frozen fruits for the continuous market.
4. Factory canned products.
5. Home canned products.
6. Wines.
7. Unfermented fruit juices.
8. Candied fruits.
9. Dried fruits.

Widened outlet for fruits is associated with pectin and this unique mat rial can increase the market for fruit enormously in two major ways. Fir pectin can be manufactured cheaply from low grades of fruit or from b products of fruit, as for example, from the pulp residues of cider and wi making. Second, pectin can be utilized for manufacture of a variety products which themselves are largely composed of fruit, as for examp jams, jellies, and marmalades.

The primary object of this bulletin is to promote interest and furni information concerning pectin.

Part I

PRACTICAL CONSIDERATIONS OF PECTIN

Chapter I

ECONOMIC CONSIDERATIONS

During recent years the State of Washington has become one of the foremost producers of fruits and berries, with world-wide markets. The chief outlets of crops are fresh fruits during season, and canned and storage products during the rest of the year. With a year-around set-up for processing the fruit crop, it is generally assumed that this industry is assured of a continued and profitable market. However, events of the past several years have necessitated a change in view point. Fluctuating markets, displacement of apparently staple commodities by cheaper but equally useful substitutes, surplus crops, increased competition, etc., are some of the factors responsible for disturbed conditions. Therefore it is no longer wise or profitable to depend entirely on former markets for consumption of fruit crops.

Two important factors have been recognized that tend to stabilize agriculture. These are diversification of products and profitable utilization of by-products. A detailed discussion of these factors, as influencing and stabilizing the fruit industry, will be given below.

Product diversification means marketing the basic material in as many forms as possible. Diversification leads to increased profits during normal times and to stabilization of prices and markets during periods of fluctuation. Stimulation of a steady demand usually brings a greater acreage under cultivation and ultimately more complete utilization of lands.

The practice of manufacturing a variety of products from one material is sound; it is the basis of success of many large industries. The Farm Chemurgic Council is advocating and successfully applying to agriculture the principles of product diversification. The history of the berry industry of Western Washington is an excellent example of application of diversification so as to yield greater profits. Several years ago, owing to lack of markets, loganberries and raspberries were sometimes left hanging on the vines. Now, because of manufacture of berry wine, these crops are contracted for months in advance. Another profitable diversification of fruit crops recently developed in this state is the frozen pack. Because frozen packs can supply fresh fruit throughout the year, or can hold the fruit for various methods of processing, they have become a most valuable factor in stabilizing our fruit industry.

Each succeeding diversification reduces the seasonal nature of the fruit industry. When the point is reached where there is no seasonal variation in sale of fruit and fruit products this industry will have become permanently stable and profitable.

By-product utilization needs no introduction. In the past few years it has become the key to profitable enterprise for many industries. In fact, it may be considered a cardinal sin to throw away by-products or waste material if Past negligence and inefficiency of agriculture in this respect is apparent; today chemical and technological studies are bringing out many useful applications of plant materials. The recent rise of the fruit-juice industry is an illustration of this relationship between technical and agricultural development. This industry owes its present success to recent improvements in the methods of commercial sterilization of liquids.

Successful development of the fruit industry should include expansion of acreage under cultivation and increased processing facilities. New mar-

kets and the facilities for serving these markets must be increased. Th this can be done has been demonstrated by the Hawaiian and the Californ fruit industries. The cooperative plan of marketing, successfully embodie in the California Fruit Growers Exchange, discloses a solution of many our problems. There is no question that the Washington fruit industry nee unification. It needs a close-knit cooperative organization capable of ser ing all interests to the best advantage; an organization backed by technic development and contr[illegible] whose integrity [illegible]nd guarantee of merchandise ar quality is unimpeachable. Until then the fruit industry will largely lac the benefits that accrue from public faith and good will.

Pectin is used largely in the preparation of jams, jellies, and marmalade It can be used to supplement the fruit juice in these products, for whic reason it is an economical asset to the preserver. By using pectin instea of fruit juice, the cost of the jelly can be materially reduced. This is e pecially true for jellies of the more expensive fruits and those deficient i pectin. Commercial jelly and jam manufacturers are able to obtain pecti sufficiently cheap to make this procedure economical. The housewife, how ever, pays from ten to twelve times more for pectin than the manufacture does when she purchases one of the retail pectin products. For this reaso home jam and jelly making is greatly curtailed. This in turn reduces th market for fruits of low-pectin content that require additional pectin t make them jell. Cheap pectin is the key to the situation. Increased produc tion of jams and jellies is accompanied by increased profits for the fruit an sugar industries of the State of Washington.

A pectin industry could be established on any of several bases. It coul be connected with some by-product source of pectin such as a cannery vinegar plant. A large source of supply is at hand and if the pomace we dried immediately and stored, a relatively small pectin plant could operat continuously to manufacture pectin products.

Establishment of a central plant for pectin manufacture would be advar tageous. For example, contracts could be made with growers or storag plants for delivery of apples into the plant or they could be purchased sul ject to delivery by the grower. Such a plant in a large apple-producir district would be assured of ample raw material and the growers would b given a market for most of their present unsalable stock. Also it is possib for individual growers to dry and ship apples to one central plant for pro essing. The cost of shipment would be reduced about 75% by drying t apples. This plan seems to be the best for all concerned as it enables ea grower to dispose of his surplus and affords the manufacturer the adva tages of a single large plant rather than duplication of several small on Such a plant could be operated profitably as a private industry, as a Sta Grange enterprise, or under a cooperative plan such as is used by the Ca fornia Fruit Exchange. The last is probably the best as it co
tages of cooperative buying, manufacturing, selling and dev
greater profits could be assured and cheaper pectin could be su
consumer.

Chapter II

COMMERCIAL SOURCES AND MANUFACTURE OF PECTIN

Commercial pectin is marketed either in the form of a concentrated solution or as a dry powder. Solutions are prepared by evaporating and clarifying fruit extracts. Powdered products are manufactured by evaporating pectin solutions to dryness or by precipitating pectin from fruit extracts.

The oldest method of pectin manufacture by precipitation involves the treatment of the fruit extract with two or three volumes of ethyl alcohol. In some cases the fruit extract is concentrated by evaporation in order to reduce the volume of alcohol needed for precipitation. Isopropyl alcohol and ethyl alcohol are approximately of the same cost; the former possesses certain qualities that make it superior to the latter for pectin manufacture, such as lower vapor pressure, and higher precipitability. Furthermore, the use of isopropyl alcohol is not subject to Federal restrictions.

Sardik (1) perfected a process for manufacturing dry pectin; the pectin extract is run onto a heated revolving drum in such a manner that a continuous thin film of dry pectin is formed. Glycerol is used to prevent the film from adhering to the drum. Another important method for manufacturing powdered pectin is the precipitation method of Jameson and Taylor (2). This process involves the formation of an aluminum hydroxide precipitate in the fruit extract, the pectin being precipitated simultaneously with the aluminum hydroxide.

Pectin from Apples

The commercial sources of pectin are citrus and apple waste materials. Apple wastes, in the form of pomace or peels and cores, are by-products of vinegar manufacture and canning operations. A typical analysis of wet apple pomace follows (3):

Wet Pomace Moisture....72.66 per cent
Solids .....27.34 per cent

	Wet Basis	*Dry Basis*
Pectin	2.62%	9.56%
Sugars (as invert)	10.50	38.52
Starch	5.01	18.35
Nitrogen (albuminoid)	0.12	0.67
Ash	0.62	2.26

The presence of lead and arsenic on Washington apples has been the chief objection to their use in pectin manufacture. Speas and his associates have worked out methods for preparing lead and arsenic-free pectin from these apples. The lead (4) is precipitated as the oxalate and the arsenic (5) is removed by means of tannic acid and ferric hydroxide. It is doubtful if these processes are completely satisfactory.

It is recognized that lead arsenate spray-residue can be removed entirely from apples by acid washing provided the temperature of the bath is sufficiently high. In usual practice, because of the harmful effect on the keeping quality, there is a limit to the degree to which the apples can be heated. This would be no objection in washing apples for use in pectin manufacture. Washing at a temperature sufficiently high to remove all the lead and arsenic would be simple and economical as a method of spray removal.

Cull apples and thinnings are potential commercial sources of pectin. The grading operation in which culls are sorted out follows the usual lead and arsenic removal process. It is estimated (6) that approximately 40,000 tons of cull apples are wasted annually in the Northwest. The pectin in these apples would furnish nearly a million dollar industry. Thinnings can also be utilized for pectin, provided they are given proper treatment (7). A profitable means of utilizing them is desirable as disposal of apple thinnings is a problem in coddling moth control.

Apple pomace from cider and vinegar operation is largely free from sugars, which is important in preventing the stored pomace from spoiling. Moreover, the presence of sugars in the final pectin solution reduces the jellying power of the product If cull apples are used directly for pectin manufacture, the sugars and foreign materials are removed by pressing out the juice and washing the pomace with cold water.

Apple pomace for pectin manufacture should be dried as it comes from the presses. This operation is important for several reasons. The drying process permits storage of the pomace so that the plant need not be designed for great capacity, and renders the pectin more soluble. The usual process is to heat at 150°-180° F. until the moisture is reduced to 6-8%. By this treatment one ton of apples yields approximately 100 pounds of dried pomace.

To make a colorless, tasteless, product, the dried pomace must be leached substantially free of coloring and flavoring matter before extracting the pectin. Rooker (8) recommends leaching with cold water until the specific gravity of the wash water falls to 1.005. The washings are utilized for vinegar manufacture.

The pectin is extracted by heating the leached pomace in several volumes of water. The acidity of the mixture should be adjusted to a pH of 2.3-4.0. If the acidity is too great the jellying power of the pectin is destroyed; if it is too low, a poor yield of pectin is obtained. Heating for a long time or at high temperature has the same effect. The following figures (9) show the effects of acidity, time and temperature on the amount and jelly grade of the pectin.

Oranges (1)

	Alcohol Precipitate, Per Cent	Acid In Extract (Calcd. as Citric), Per Cent	Sugar In Jelly, Grams	Extract Used In Making Jelly, Grams	Grams of Jelly Per Gram of Extraction	Grams of Jelly Per Gram of Peel
Open kettle, distilled water...	0.347	0.070	84	70	2.0	5.2
Open kettle, 0.1 N acid.....	0.890	1.900	84	27	5.2	13.7
Autoclave, 5 lb., 30 min., distilled water	0.565	0.057	84	90	1.5	3.9
Autoclave, 5 lb., 30 min., 0.1 N acid.....	0.960	1.270	84	60	2.3	6.0
Autoclave, 20 lb., 30 min., distilled water	1.690	0.120	84	90	No jelly	
Autoclave, 20 lb., 30 min., 0.1 N acid.....	1.770	1.520	84	70	No jelly	

Apple Pomace (2)

Time of Cooking (hours)	½	1	1½	2	
Pectin (%)	1.17	1.45	1.46	1.43	Acidity=0.10% (fruit acid)
Pectin	1.26	1.30	1.57	1.73	Acidity=0.24% (fruit acid)

According to Rooker (10) the best method for extracting pectin from apple pomace comprises addition of three parts of water to the pomace, adjustment of the pH to 3.5 and heating at 190° F. for 1-1½ hours. If the extraction is carried out at 212° F., only 30-40 minutes heating is required.

Because the apple pomace is disintegrated by the extraction process, the pectin liquor is prevented from draining out by gravity alone. Separation is best accomplished by pressing while hot. The press-juice is clarified by natural settling, mechanical precipitation, or by centrifugal methods. The last process is the most economical because it reduces sludge losses, time, and space requirements. The juice is centrifuged while hot and is then cooled to 120°-125° F.

Notwithstanding washings and leachings, the pomace still contains objectionable materials such as proteins, starches and sugars. These affect the clarity and jellying power of the pectin product and should be removed. The most effective method for eliminating these impurities is by treatment with diastatic (11) and proteolytic enzymes (12). The enzyme preparation is dissolved in water and poured into the pectin solution. The action of the enzymes is complete in 30-60 minutes at 120° F. Some enzyme preparations also contain pectic enzymes that must be destroyed in order to prevent the loss of pectin. This is accomplished by heating the pectin liquor to 170° F.

Decolorizing charcoal is added at this point and the temperature is maintained at 170°-180° F. until control samples indicate complete decolorization. Following this the temperature is reduced to 140° F. and the liquor is clarified by passing through a filter press.

The clarified pectin solution is concentrated by evaporation, preferably in vacuum pans. Glass lined or block tin rather than copper, aluminum, or iron kettles are used. The pectin solution is cooled, adjusted to the proper pectin and acid concentrations, and bottled. Preservation is effected by pasteurizing at 170° F. for thirty minutes.

Dried pectin products can be prepared by several methods: by treating the clarified pectin solution with alcohol, separating and drying the pectin precipitate in vacuum; by spray-drying the pectin solution; by precipitating the pectin from solution by the action of aluminum hydroxides, etc.

Patent Situation

At present the only valuable processes for pectin manufacture covered by patents still in existence are the methods of precipitation by aluminum hydroxide (2) and calcium (13) and the method of drying in a thin film (1). The aluminum method is probably the most economical. It is used in the manufacture of pectin from citrus fruits. The calcium process is of doubtful value because of the loss in jellying power involved.

The expired Douglas patent (14), was the one originally issued for pectin manufacture, and caused much litigation between canners and pectin manu-

facturers. It disclosed the process of manufacturing concentrated pectin solutions by evaporation of fruit extracts. The process of clarification of pectic solutions by the use of diastatic enzymes (15), now is likewise public property.

The Douglas patent (16) covering the "short boil" process of preparing jelly has expired. This process also caused litigation between canners and pectin manufacturers several years ago; the court ruled that it covered the process of reducing the boiling time by the addition of a pectin solution.

The use of dried pectin for making jelly is complicated by the fact that pectin tends to clump when it is added to water. This clumping is an undesirable condition because it takes too long a time to dissolve the pectin. Numerous patents, (17), (18), have been issued to cover dry pectin products that contain dispersing agents. These are controlled for the most part by one concern, making it difficult for others to enter the field.

Summarizing the patent situation, the methods of precipitation by alcohol, concentration by evaporation, and clarification by diastatic enzymes are available to the public. The patents on aluminum hydroxide precipitation and clarification by proteolytic enzymes do not expire for several years.

Other Sources of Pectin for Commercial Manufacture

The possibility of utilizing waste plant materials, other than apples and citrus rinds, for pectin manufacture has not been investigated sufficiently. The pectic substance of sugar beets (18) has been studied (19), (20), (21), as a commercial source of pectin. Whether this pectin possesses sufficient jellying power to be suitable for a commercial product is not certain although evidence does not seem favorable.

There has been considerable interest in grape marc as a commercial source of pectin. Mehlitz (22) reports pectin in grapes but states that grape marc does not yield pectin of commercial grade. Similar results were obtained by Marsh and Pitman (23) for California grapes. Experiments carried on at the University of Washington indicate that under certain conditions grape marc does provide a suitable commercial source of pectin (24).

Furthermore, experiments along the line of pea-hull utilization show this material is especially promising for pectin manufacture. A single acid extraction of pea-hulls yields an alcohol precipitate of 1.2 per cent. The precipitate dries and gives without further purification a white, tasteless product. The jelly grade is above 180, comparing favorably with other pectins now on the market.

In this state at the present time there is an enormous annual production of pea-hulls that is disposed of as fodder. Most of the pea crop is harvested vines-and-all, but much of it is hand picked. The latter is a good source for pectin.

Uses for Pectin

Pectin is used chiefly in the preparation of jams, jellies, marmalades, and similar products. Because there is no existing substitute for it, pectin has always commanded a good price. This high cost has discouraged other uses when, as a matter of fact, it possesses unique properties that would make it valuable for many other uses.

Pectin is a complex carbohydrate and possesses some of the properties of other important members of this class (gums, starch, and cellulose). It is

similar to gums and mucins. It can be changed to them by certain treatment, hence can be used in glues and mucilages (25), (26). Pectin is superior to starch for sizing textiles (27). Pectin extracted from residues in beet sugar manufacture, and applied to fibers, produced fibers that were as strong and as easily worked on the looms as those treated with starch. Furthermore, sizing with pectin was much cheaper and did not necessitate, as is the case with starch, the employment of auxiliary substances such as tallow, or glycerine. Pectin, like cellulose, has long filament structure. Nitro, acetyl, and formyl pectins can be prepared (28) by methods similar to those used with cellulose. These pectin compounds can be used for explosives, lacquers, etc., in the same manner as nitrocellulose and cellulose acetate.

Pectin is an excellent emulsifying agent for gases and liquids. It increases the foaming power (29) of gases in water to a greater extent than casein. Oil-water emulsions can be stabilized by pectin and, although it is effective for emulsions of the type of benzene (30) in water, it is of particular advantage in pharmaceutical and food emulsions. Because of its edibility it makes a good emulsifying agent in such preparations as cod-liver oil (31), mayonnaise, and essential oils. Pectin is also used in salves, in nasal and vaginal jellies, in bandolines, waveset, and hair preparations, and in hand and face lotions (32). In all of the aforementioned products, pectin makes a better stabilizing agent than gum acacia or tragacanth.

Pectin increases the quality of process-cheese by increasing its water-holding capacity (33). Clinical experiments (34) show that cheese containing pectin is improved in consistency and digestibility and equals that of the finest cream cheese.

Pectin is used in pastries (35) and dehydrated juices (36). Bakery products containing pectin retain their moisture and become stale less easily (37). Pectin also improves the texture (38) and yield of bakery goods when incorporated in the mixture to the extent of 5 per cent of the flour used.

Cheap Pectin

The main obstacle to large scale development of the pectin industry is its high cost. This high cost curtails its use for any other purpose than jelly making. Even the demand for it for jellies is but a fraction of what it would be if the pectin were cheaper.

A multitude of new uses and new products utilizing pectin could be developed; when a material is sufficiently inexpensive it enters into many new applications. The unique properties of pectin make it unusually certain of such success.

To place on the market cheap pectin, the manufacturer must change over to mass production with a smaller margin of profit. Substantial profits would not be sacrificed thereby and greater utilization of fruit and vegetable wastes would result.

Also cheaper processes could be developed in which jelly power would be sacrificed for economy of productions. Pectin of this type would find many uses where jellying power is unimportant. For example, large quantities of pectin of low jellying power are available from exhausted sugar beet pulp and similar by-products. This pectin still retains most of its characteristic properties and could be used in many ways other than in jelly making. Cheap processes for obtaining this pectin can be developed.

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Chapter III
JELLY MAKING

The earliest published record of jelly making appeared in the latter part the eighteenth century. Since then, furnishing as they do a delightful d that possesses fresh fruit flavor and odor, jellies have become a very portant item in the daily diet.

It was not until early in the nineteenth century that pectin, the agent using jellying of fruit extracts, was discovered. Since then much study as been made of its jellying properties; however, the operation of jelly aking has remained a highly empirical art, with successful production rgely depending on the skill of the individual.

It is not the purpose to devote this chapter to recipes for jelly making ut rather to consider the factors involved in its formation. These will serve s a guide in understanding jelly making, the problems involved, the causes failure, and the like.

he Necessary Components of Jelly

Pectin is the essential material in jelly compositions. Before a fruit juice ill jell it must contain from one-fourth to one per cent of pectin. Lesser oncentrations cause jelly failure; greater concentrations cause premature jellying and tough jellies. Some fruits possess much pectin, but because pectin can exist in non-jellying forms, these fruits may fail to yield jellies. In fruits that are known to possess jellying capacity, the quality of the pectin varies with ripeness. This explains why slightly underripe fruits rather than green or overripe fruits make better jellies.

The jellying pectin is easily changed to a non-jellying form by heat, acids, plant ferments, molds, and bacteria. Therefore substantial avoidance of these adverse conditions and materials must be made. The jelly should be heated no longer than is necessary; it should be cooled quickly thereafter. High acid fruits, such as currants, should be mixed with water or with other fruits of low acidity. Plant ferments act rapidly on the pectin in pulped or bruised fruits; these enzymes should be destroyed by heat.

Jelly is the product of a favorable combination of pectin, sugar, acid, and water. Formation of the jelly occurs only when the ingredients are present in certain concentrations, as indicated approximately by the percentages in the following formula:

Sugar	60 to 65 per cent
Water	33 to 38 per cent
Fruit acid	1 per cent
Pectin	1 per cent

A detailed consideration of each of these factors will be taken up in this chapter.

Many fruits contain sufficient pectin for jelly making as for example, grapes, apples, blackberries, currants and loganberries. Other fruits as strawberries and raspberries require the addition of pectin. This deficiency of pectin can be compensated in two ways, either by addition of juice or extract of material high in pectin, or by treatment with a commercial pectin product. Each of these has advantages and disadvantages. The addition of another fruit extract is less expensive but influences the flavor of the jelly. It also requires special control of mixing in the proper proportions to form jelly. Less difficulty is encountered by using a commercial pectin product; it is only necessary to add a quantity of the pectin syrup or powder calculated to give a jelly. It is claimed that commercial pectin decreases molding.

Commercial pectin products are obtainable in both liquid and solid forms and are standardized for sugar carrying capacity. A statement of 160 grade signifies that one pound of the material is capable of causing the jellification of 160 pounds of sugar. Usually the liquid products are obtained by vacuum evaporation of fruit extracts, followed by purification and clarification. These do not always yield the colorless, tasteless, and odorless products desired in very delicate jellies. On the other hand, the solid powdered products are satisfactory as to color and flavor but are difficult to dissolve. A decided advantage of powdered material is that it does not deteriorate by exposure to air.

Acid

The acid content of the juice is of great importance. Jelly will not set unless it contains a certain quantity of acid; too much acid will cause the jelly to weep or run. A simple method for estimating the available acidity follows: compare the tartness of the fruit juice with that of a solution of one teaspoonful of lemon juice and one-half teaspoonful of sugar in nine teaspoons of water. If the fruit juice is less tart than the lemon juice, acid materials, such as lemon juice, vinegar, tart fruit juice, tartaric or citric acid, must be added. If the juice is more tart it must be diluted.

Sugar

The third important factor in jelly formation is the sugar concentration. When too much sugar is used, the jelly is too fluid; when too little sugar is used the jelly is tough. It should be understood that either beet or cane sugar can be used. The sugar should be added when the juice begins to boil. The jelly must be boiled down to a 60-65% sugar concentration. Juices low in pectin require longer boiling than high pectin concentrations. Because it is desirable to limit the time of heating, the sugar should be heated before adding to the boiling juice. The practice of adding the sugar after the juice is boiled down is not good because a crystalline jelly may result, the sugar does not completely dissolve and it crystallizes on cooling and standing. Sugar inversion and deterioration of the jelly may result, if heating is continued too long. In addition to its importance in the formation of jelly, the sugar serves as a preservative. Jelly containing less than 65% sugar is liable to undergo yeast fermentation or mold decomposition.

Water

The fourth important component of jelly is water. It should be varied so as to give the proper concentration of pectin, acid and sugar; its adjustment should substantially conform to the above formula. If the acidity is high, water can be added or the juice can be diluted by addition of other juices containing little acid. The proper combination of the four components requires all the skill of the jelly maker.

Requirements of Fruits Used in Jelly Making

The choice of fruit to be used involves many considerations some of which are overlooked by the housewife. Even some commercial manufacturers of jams and jellies are careless in this respect. With the latter there may be a lack of knowledge or a short-sighted policy whereby quality is sacrificed for greater profits.

Ripeness

The best flavored jellies are obtained from ripe, sound fruits. However, other factors may influence choice. For example, if tartness or greater jelling power are sought, greener fruits may be used. On the other hand, because of their superior color and flavor, over-ripe fruits may be used. These may be low in jellying power and will require addition of some material of greater pectin content such as other greener fruits, or a commercial pectin.

Over-ripe fruits have lost much of their original jelling capacity because of natural enzyme action, or because of mold and bacterial action. Other factors causing enzyme changes in fruit are the length of elapsed time and the treatment after picking.

It was pointed out in the chapter on pectin enzymes that pectin degradation proceeds rapidly after picking. Obviously these changes must be avoided by the jelly maker. However, the fruit must not necessarily be picked ripe and used immediately. The ripening process may be promoted in storage. Hastening the pectin development to an optimum point has been employed with apple thinnings. These possess no jellying power originally, but, by macerating and allowing them to stand under hydrolysing conditions, a satisfactory product can be obtained in one or two days. Obviously the technique of jelly making is directed toward a maximum concentration of active pectin. Closely allied to the age of the fruit after picking is its after-treatment. The material is easily bruised or crushed and, if it is carelessly handled, destruction of cell structures may promote rapid degradation. The temperature of storage is also of great importance. Lower temperatures inhibit metabolic changes.

The following shows qualities of various fruits for jelly making, as based on pectin and acid contents:

I FRUITS RICH IN PECTIN AND ACID	II FRUITS CONTAINING LESS PECTIN & ACID	III FRUITS RICH IN PECTIN, BUT LOW IN ACID	IV FRUITS CONTAINING ACID BUT LOW IN PECTIN	V FRUITS LOW IN PECTIN AND ACID
Apples, sour and crab	Apples, ripe	Apples, kinds low in acid	Apricots, sour	Apricots, ripe
Blackberries, sour	Blackberries, ripe	Bananas, unripe	Cherries, sweet varieties	Elderberries
Cranberries	Cherries, sour varieties	Cherries, sour	Peaches, sour	Peaches, ripe
Currants	Fejoias	Figs, unripe	Pineapples	Pomegranates
Gooseberries	Gr'pes, California	Pears	Rhubarb	Prunes
Grapes, eastern	Loquats	Pie melon	Strawberries	Raspberries
Guavas, sour	Plums	Grapefruit, peel		Strawberries
Kumquats		Guavas, ripe		Overripe fruits
Loganberries		Oranges, peel		
Lemons		Quince, ripe		
Oranges, sour				
Plums, sour				

I Make jelly easily.
II Make jelly if care is exercised.
III Require addition of acid or acid fruits.
IV Require addition of pectin or fruit rich in pectin.
V Require addition of pectin and acid.

EXTRACTING THE JUICE

It is usually necessary to boil the fruit with water to extract the pectin and destroy the enzymes. Loganberries, currants and cranberries need not be cooked because their juices contain sufficient pectin to form jelly. If these juices are pressed out and immediately made into jellies, their pectin is not appreciably affected by the enzymes. In most cases, however, the insoluble pectin in plant tissues does not dissolve unless heated. In the fruits mentioned the mass of jelly that can be made from a given quantity of fruit can be increased by adding a certain quantity of water and heating for two to three minutes.

Apples require addition of one or two cups of water per pound of fruit and boiling for fifteen to twenty minutes. Quince, citrus, and other hard fruits must be finely sliced and heated for thirty to sixty minutes with twice their weight of water.

The ideal quantity of water to be used is that necessary to give juice containing sufficient pectin and acid to make the jelly. When too little water is used, there is danger of scorching the fruit or obtaining cloudy and viscous juices that are difficult to make into clear jelly. Diluted juices must be concentrated by continued boiling but only so long as to give the jelly test. When using fruits high in pectin, such as loganberries, cranberries, currants, etc., it is better to make two or three extractions with small portions of water and later to combine the extracts, rather than make a single extraction with a large quantity of water.

The fruit should be heated for a limited time, so that the juice can be pressed out as completely as possible. When heated too long, it becomes mushy and fine particles pass through the filter cloth. This suspended material is very difficult to remove and causes cloudy jelly. Little or no loss of the delicate fruit flavor and aroma will result if the period of heating is short.

Copper and iron kettles can cause darkening of the color of the juice. Aluminum is satisfactory if the fruit is not too acid, and the pan is kept clean. The best materials are glass, unchipped graniteware, or enamelware.

The juice is separated from the pulp by straining through cloth bags. These can be made of linen, flannel, felt, or folded cheese-cloth. Linen and cheese-cloth are preferred because of their durability. The bag should be scalded and used while still wet or moist, and cleaned thoroughly when finished. To obtain the juice, let it drip or squeeze it through the bag. The first method yields clearer juice; pressure will give good juice if strained through the bag several times. To obtain a clear juice in a simple manner, squeeze it through a cheese-cloth bag and then allow it to drip without pressure through a linen or hair-cloth bag. The following is a simple method, combining extraction and expression: Place the jelly bag containing the sliced or crushed fruit in boiling water and allow to remain for twenty to thirty minutes. A part of the juice is then gently squeezed out of the fruit and the remainder is allowed to drain out without pressure.

Cooking the Jelly

Boiling promotes union of the jellying components and concentrates the solution to the jelly state. It is desirable to concentrate the mixture as rapidly as possible. The quantity of juice that can be handled at one time will depend on the heating facilities, the size and shape of the kettles, and the

skill of the operator. No more juice should be taken than can be boiled down in about twenty minutes. If the equipment and heating facilities are sufficient to handle large batches of jelly at one time, the size of the batch should not be greater than can be poured and sterilized with ease.

To determine the best ratio of juice and sugar to use, make a preliminary run on a single cup of juice and a medium quantity of sugar. Boil until the jelly test is given, and then pour. After it has cooled, observe the quality of the jelly. If it is thin, too much sugar has been added, if it is tough and stiff, too little sugar has been added. Skimming the hot juice at intervals will yield superior jellies.

Testing For the Finished Jelly

The end-point of the boiling process can be determined by the "sheeting" test, or "paddle" test, or by use of a jelly thermometer. For example with apples, when the temperature reaches 221° F., the juice has attained the desired concentration of 65% of sugar. The temperatures for current and grape jellies are 223°-225° F., and for guava jelly, 226° F. It must be remembered that at altitudes higher than sea level, the boiling points are correspondingly lower. At any altitude the end-point will be about eight to ten degrees Fahrenheit above the boiling temperature of water at the same altitude. The end-point of the boiling process depends also on the firmness desired in the finished jelly. Jellies for home use need not be as stiff as those designed for shipment.

A more common method for determining the end-point of boiling is by the "sheeting" test. Some of the juice is taken on a large spoon or wooden paddle and is allowed to run off the sides. At first it drips off like syrup; the drops then become heavier. When the liquid no longer falls off in individual drops, but runs off the paddle as a sheet, the jelly is done and should be skimmed and poured immediately. Successful application of this test depends on the skill of the operator. Once mastered, it gives satisfactory results.

Pouring the Jelly

The jelly should be poured immediately into hot, dry, sterilized glasses. The glasses should have smooth sides so that the jelly can be turned out in one unbroken mass. In order to prevent the glasses from breaking while pouring in the jelly, it is advisable to pre-heat them. Pouring through cheese-cloth removes scum and solid material. Rapid cooling yields better jellies. If the jelly is weak or does not set, the glasses can be heated in a pan in the oven. Covering them with a pane of window glass and setting them in the sun may promote jellying. In this way excess water can be evaporated without deterioration in color and flavor.

Sterilizing and Sealing the Jelly

The purpose of sterilizing and sealing jelly is to prevent growth of yeasts and molds during storage. Such precautions are especially necessary when the sugar concentration is less than 65 per cent.

Ordinarily, it is sufficient to pour a thin layer of hot melted paraffin onto the hot jelly and then set aside. As it cools, however, the paraffin film may pull away from the glass and, unless another layer of hot paraffin is added, the jelly is liable to spoil at the fracture. A good seal can be made by running a pointed stick around the edge while the paraffin is still hot.

Another method of sterilizing is to dip a circle of paper, cut to fit into the glass, in alcohol or brandy, and place it on top of the jelly. The alcohol kills the organisms present on the surface. After sealing the jelly with the circle of paper or with paraffin, the jelly should be protected by a paper or a tin cover. This is necessary because the seals are not always efficient in keeping out mold spores.

Even these precautions may fail when the sugar concentration is too low to inhibit the growth of microrganisms. It then becomes necessary to follow the practice of pasteurizing the sealed jelly by immersing in simmering hot water for about fifteen minutes.

Jelly should be stored in a cool, dark, dry place. Light causes the color to fade and may cause the jelly to weep. Long storage results in slow deterioration in color, flavor, and texture.

Jellies Without Cooking

Such fruits as apples, loganberries, currants, and cranberries are so rich in pectin and acid that jellies can be made from them without heating. Sugar is added in the proportion of one and one-half cups to one cup of juice and stirred until dissolved. The mixture is poured into glasses and allowed to stand in the sun until it sets into a jelly.

Fancy Jellies

Fancy jellies can be made from fruits that contain little or no pectin. by using commercial or home pectin preparations. Mixtures of fruits or flavors such as mint or ginger can be used. Thus numerous pleasing combinations and delightful preparations can be made.

Layered jellies, consisting of several layers of different color and flavor. can be prepared by pouring a layer of one kind and allowing it to set, pouring the next layer of a different kind of jelly, and allowing it to set. and so on. Placing a rose or a geranium leaf in the glass while pouring the jelly adds to the flavor and appearance.

Jelly Stocks

Fresh jelly can be made at any time during the year by following the practice of putting up fruit juice in jars or bottles and making the jelly as it is needed. The boiling juice is poured into hot, sterilized containers and sealed immediately. This is stored and whenever convenient is used in the usual manner for making jelly.

Causes of Jelly Failure

Jellies may be too soft in texture for several reasons. Too much sugar was used; the jelly was not boiled long enough; or too little acid was present in the fruit juice. Tough jellies usually result from deficiency of sugar. Juices high in pectin jell even though sufficient sugar is not added. but the jelly will be tough. Prolonged boiling after the jellying point has been reached will also give tough jellies. Cloudy jellies result from careless straining of juice. Cooking until the fruit becomes completely pulped usually gives cloudy, starchy jellies.

Ordinarily crystalline jellies result from excess of sugar. Jellies will hold about 70% of sugar before they begin to crystallize. Owing to slow conversion of sugar into a more soluble form, continued boiling may increase the quantity of sugar that jellies will hold. Sugar added after the juices have

been concentrated cause crystalline jellies; addition of sugar when the juice first boils will prevent this effect.

Cream of tartar crystals may appear in grape jellies. Concentrating the juice by boiling and allowing it to stand until cream of tartar settles out is advisable. The juice is then strained, diluted, and used for jelly.

JAMS

Jams and jellies are made by like processes, except that with jams the fruit pulp remains in the finished product. Any fruit or combination of fruits can be used; small fruits and berries are commonly used. Their jelly-like texture results from proper concentrations of pectin, fruit acid, and water. In making jams it is necessary to observe all conditions controlling jelly making. However it is possible to reduce the quantity of sugar used in jams without impairing the quality of the product. For berries, three-fourths of a pound per pound of fruit is satisfactory, although the pound-for-pound ratio can be adhered to. With some of the sweeter fruits, especially with those of low acidity, such as ripe peaches, sweet grapes and prunes, the sugar must be reduced to one-fourth of a pound for each pound of fruit. More sugar and water can be mixed with the pound of fruit by adding pectin. Then the same technique of boiling and testing used in making jellies should be employed.

Perfect or imperfect unspoiled fruit can be used for jams. A product possessing the flavor of the fresh fruit and the jellying property of green fruit can be obtained by combining these in equal proportions.

The fruit should be cooked until it becomes tender and the juice gives a good test for pectin. Rapid cooking with constant stirring is necessary. Sugar is added and the cooking is continued until the jellying point is reached. Excessive boiling must be avoided before the sugar is added. Otherwise the mixture jells prematurely, and breaks up on pouring into the container. Such jam is also liable to crystallize because of the high sugar content.

MARMALADE

Marmalades are essentially jellies that have pieces or slices of fruit suspended in them. The similar conditions in respect to pectin, acid, and sugar concentration, required in jelly making, apply to marmalades. To make marmalades, the pectin and acid contents of the fruits must be higher than in jellies and jams.

Some of the fruit is cut up and boiled, and the juice is strained off to give a pectin solution. Another portion of the fruit is then sliced and cooked until done, and added to the pectin solution. Sugar is added pound-for-pound, and the mixture is boiled until the jellying test is obtained.

Marmalades are usually made from citrus fruits, such as oranges, grapefruit, lemons, and kumquats. The pectin is obtained from the white portion of the rind. The shredded peel is first extracted with boiling water to remove bitter flavor, usually four or five extractions being sufficient. The pectin solution can then be prepared in one of two ways, either by the three-day or the single-day method. The quality of the product obtained is the same for either process, although a greater yield of marmalade can be obtained by the three-day method. The washed and sliced fruit is permitted to stand

overnight in three times its weight of water. The next day, it is boiled for thirty minutes and allowed to stand again for twenty-four hours. Then the fruit and juice mixture is weighed and sugar is added in the ratio of one pound of sugar to every pound of juice. After boiling until the jellying point is reached, it is cooled to 175° F. and poured into containers. The cooling is necessary to bring the marmalade to the jellying point, so that the fruit will not settle out when poured.

In making marmalade by the short method, the peel is sliced and the bitter substance is extracted, the slices covered with water, and boiled until tender. Meanwhile the edible portion of the fruit is boiled with twice its weight of water. The juice is strained from the disintegrated pulp and added to the mixture containing the shredded peel. To each pound of this combined mixture is added one and one-half pounds of sugar and the marmalade is boiled until the jellying point is reached. The boiling process should be completed in about twenty minutes. Limited boiling produces bright, sparkling marmalades.

COMMERCIAL PRODUCTION OF JELLY

Jelly is made commercially in the same manner as in the home. The discussion in the previous sections concerning fruits, pectin, acid, and sugar requirements, methods of extracting and filtering, boiling and packaging, etc., are applicable to large scale manufacture. The following section will be devoted to special problems.

Obtaining the Juice

The juice is extracted by boiling in kettles of 50 gallon capacity or greater; the kettles are so installed that juice can be drained off by gravity to the clarifiers. Heating is accomplished by steam-jacketed kettles composed of material that is not attacked by the fruit acid, such as glass, monel metal, tin, silver-lined copper, etc. Acid-resistant steam coils are installed in glass kettles for more efficient heating. The usual types of clarifiers are used. Pulp filters and filter presses give fair results. Filter aids are used in filter presses with good results. High speed centrifugal clarifiers are rapid and efficient. The juice is first freed from coarse, easily removed material and is then run through the centrifuge. This makes clarification a very simple and inexpensive operation and yields a brilliantly clear product.

Two or even three extractions of the fruit can be made, depending on the quality of the jelly desired and the quantity of pectin and flavor left after the first extraction.

Boiling

Prolonged boiling yields jellies of poor flavor and color and sometimes so reduces the jelly power of the pectin as to cause failure. For this reason it is recommended that small kettles be used. These may be of the same type as is used in the extraction of the juice, and should not be filled too full. Skimming should be made during boiling to remove the coagulated proteins and other organic materials. The end-point of the boiling process can be determined in the usual manner. If the jelly is stabilized by pasteurization, lower final concentrations than 65% of sugar can be used.

Pouring and Preserving

The jelly should be cooled before pouring into glasses. This is accomplished by stirring in shallow, water-cooled pans, and when the temperature reaches the desired point the jelly is poured into glasses and sealed. If the jelly is to be pasteurized, it is heated to 180° F. Sealing is accomplished by means of the vacuum seal. A metal cup with a rubber ring is attached to the jar. During pasteurizing the rubber softens and forms an air tight seal at lowered temperatures. Sometimes jelly is put up in tin cans or in tubs. The tubs are sold to restaurants, and bakers, etc.

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Part II

SCIENTIFIC FACTS CONCERNING PECTIN

Chapter IV

PECTIC MATERIALS OF PLANTS

The role of pectin in plants was first recognized by Payen (1) who found that it acts as an intercellular cementing material. Mangin (2) confirmed this finding and showed that the pectic materials of the middle lamella and the primary cell-walls differ, only the latter being soluble in dilute acids. However, since it is soluble in ammonium oxalate or alkaline solution, he suggested that it is calcium pectate. This calcium salt is known to be insoluble in dilute acids and to be quite concentrated in the middle lamella (3) (4). Nevertheless, the work on the occurrence of pectates in the middle lamella is of little value because the effects of enzymes were not taken into consideration. Unless destroyed, these enzymes rapidly cause formation of calcium pectate, and, whereas the original pectic materials of the plant may not have contained pectates, the enzymes may form them, even during the course of the analysis.

Clark (5) has shown that protopectin occurs as cementing substance in cellulose fibers. According to X-ray studies the macro-mols of cellulose fibers are composed of bundles or groups of tetra-glucosan units. The protopectin occurs as a non-birefringent coating to these bundles. This view is also supported by the researches of Fahr and Eckerson (6). On the other hand, the experiments of Van Iterson (7), Frey-Wissling (8), and Bonner (9), have shown that the macro-mols of cellulose are not cemented by pectin but are held by intra-molecular forces of the cellulose molecules. The protopectin forms a chain-like network of intercellular material that meshes in with the cellulose structure.

Buston (10) states that pectic materials develop under conditions of rapid growth and high water content. This is evidenced by the presence of large concentrations of pectin in fruit, stalks of fast growing plants, the spring wood in trees, etc. The research of Gaddum (11) shows that pectic materials function in the translocation of water in the plant. This follows from Reed's theory (12) that all hydrophilic colloids of the middle lamella and the cell-wall serve as means of such translocation. Thus, the direction and the rate of movement of water between different points in the plant is governed by their difference in water-absorbing capacity. Also it is seen that the natural hydrolysis of protopectin and pectin serves as the mechanism for the movement of water. The areas where the protopectin has not been hydrolized possess a greater water absorbing capacity, hence water flows to these areas.

The pectic changes occurring during ripening of apples was studied by Carre (13). She observed in the early stages that there was no soluble pectin but during ripening the content of soluble pectin increased. The maximum content of soluble pectin was reached when the fruit was fully ripe. Later there was a steady decline in pectic components. In studying the natural changes in pectin during storage, Carre (14) found that the sum of the concentrations of protopectin, pectin, and pectic acid remained constant until May. During the last four months pectic acid was formed from pectin as rapidly as the pectin was formed from protopectin. At the end of the eighth month the pectic acid itself began to break down.

Staining studies of ripening apples (15) with ruthenium red showed no pectin in the unripe fruit. As ripening continued, staining became apparent

on the margins of the cell wall with intercellular spaces showing increase of pectic material. The stained portions gradually extended in a continuous zone that was not dissolved by Schweitzer's reagent. Over-ripening is accompanied by weakening and complete dissolution of the framework. It was found that the staining characteristics of the natural pectic changes occurring during ripening could be duplicated by hydrolysis with water, acid or alkali.

Tetley (16) found that isolated pectic acid and pectates but not pectin are stained by ruthenium red. She concludes that plant tissue that takes this dye is not protopectin or pectin but pectates and pectic acid. Also, since ruthenium red stains other materials such as oxy-cellulose, iso-lichenin, and glycogen, it cannot be considered specific for pectin (17).

On the grounds that pectin is not found in lignified tissue, and lignin is not found in pectinous materials and that pectin disappears as lignification proceeds, it is argued (18) that pectin is the precursor of lignin in plant tissue. Ehrlich (19) was able to prepare a lignin-like substance from flac pectin by chemical treatment. He summarized other evidences for believing pectin to be the forerunner of lignin as follows: (a) wood contains practically no pectin; (b) the larger part of the lignin is found in the middle lamella of wood, analagous to the pectin of the new nourishing tissues of young plants; (c) it appears that the methoxyl and acetyl content of lignin is derived from pectin; (d) in addition to containing galactose, all kinds of pectins are characterized by a high content of pentoses or pentose-like groups such as arabinose, xylose, and tetragalacturonic acids (which can be regarded as carboxyl pentosans); and (e) it is very probable that conversion of pectin into lignin is caused by chemical and enzyme processes during growth and aging. The change can be represented by the equation:

$$C_{46}H_{38}O_{40} = C_{45}H_{18}O_{16} + CO_2 + 10H_2O + 60_2$$

Buston (20), however, does not believe lignin to be a product of pectin change. He concludes that pectic substances are produced in fast growing plants; that slower growing plants predominantly contain derivatives of glucose, as for example, glucosan, xylan, and xylose; that oxidation and decarboxylation of glucosan to xylan take place rapidly, because glucuronic acids are very rarely found in appreciable quantities. However, it is quite possible that certain plant hemi-celluloses are derived through the intermediate step of pectin formation. On chemical treatment pectin is known to yield (21) (22) such hemi-celluloses as arabans, galacto-arabans, etc. O'Dwyer (23) concludes that lignin is formed from pectin through the intermediate state of hemi-cellulose formation.

Due to its cementing action pectin maintains the rigidity of the plant structure. In addition pectin plays an important role in the break-down of this structure. Because of the hydrolytic action of the plant enzymes and acids, pectin is slowly destroyed. This allows the cell material to slough off where it is readily decomposed by mold and bacterial action. It is interesting that the luxuriant growing, perennial plants possess pectic binding material. If the plants had a more resistant binding agent such as lignin, the accumulation of decaying plant life would be enormous. Pectin also occurs particularly abundantly in the pericarp where its function is evidently to bring about the rapid release of the seeds after the period of ripening.

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Chapter V

PROPERTIES OF PECTIN

Nomenclature and Definitions

Pectin has been of great interest since the time of Braconnot (1), the first investigator to establish its role as the jellying principle of fruits. Because pectic substances and their derivatives are complex and difficult to characterize, much confusion exists in regard to their structural compositions. Their complexities and also the variety of their products of hydrolysis have developed a voluminous and confusing literature, complicated by the tendency of each worker to use his own system of nomenclature. Adoption of the following nomenclature recommended by the Agriculture-Food Division of the American Chemical Society (2) has lead to clarification of the literature and stimulation of further studies of pectin.

Pectic Substances

"Those complex carbohydrate derivatives which occur in plants, or are prepared from plants and which are characterized by the presence of galacturonic acid units. In the naturally occurring substances these galacturonic acid units apparently exist in an acid reacting complex associated with arabinase and galactase units. This acid complex may occur as a free acid or as a metallic salt, but usually as a methyl ester."

Protopectin

"The term applied to the water-insoluble, unhydrolyzed pectin substances in the state in which they occur in plant tissues. Protopectins are rendered soluble by treatment with enzymes, acids or certain other reagents, whether by peptization or hydrolysis not being known. The substances thus rendered soluble are designated collectively as pectin; it is not known whether or not the pectins are different chemical individuals from protopectins."

Pectin

"The term applied to the water soluble, methylated pectin substances occurring in plant tissue or to the methylated pectic substances obtained by restricted treatment of protopectin with protopectinase, acids or other reagents, the treatment being so regulated as to produce maximum solution of pectic substances with a minimum cleavage of methyl ester groups. The product may be a mixture of substances of varying methyl ester content; the term pectin or pectins is accordingly a group designation for all intermediate pectic substances between protopectin and pectic acid. It is proper, however, to refer to an individual of the group as pectin."

Pectic Acid

"The term applied to the pectic substances obtained by the hydrolysis of pectin with the complete elimination of the methyl ester groups. Pectic acid may be variable in composition according to the type of hydrolysis employed; accordingly there may be a number of pectic acids, all of which are ester free."

The various authors in the past have used these and other terms in several senses. Following is a table, which may serve as a guide in reading,

showing the terminology used by each worker and the equivalent in the preferred nomenclature.

A. C. S. Terms	Pectic Acid	Ca-Mg salts of pectic acid	Pectin	Protopectin
INVESTIGATOR				
1. Braconnot (3)	Gallertsäure	Pectic acid salts	Pectin	Pectin
2. Mulder (4)		Ca pectate pectin		
3. Fremy (5)	Pectic acid Pectin Metapectin Parapectin	Pectose Protopectin Ca pectinate	Pectin Parapectin Pectosic acid Pectic acid isomer	Pectose
4. Chodnew (6)	Pectic acid Perpectic acid		Pectinic acid Pectosic acid	
5. Payen (7)		Ca pectate		
6. de Haas and Tollens (8)	Pectin	Pectin	Pectin	Pectin
7. Mangin (9)		Pectose		Protopectin
8. Caldwell (10)		Ca pectate		Pectose
9. F. Ehrlich (11)	Tetra-galact-uronic acid		Pectic acid Hydrato pectin*	Protopectin
10. Tschirch (12)	Cyto-pectic acid	Protopectin		Pectose Protopectin
11. Fellenberg (13)	Pectic acid		Pectin	Pectose Protopectin
12. Onslow (14)	Pectin		Pectinogen	Pectocellulose
13. Clayson, Norris and Schryver (15)	Cytopectic acid	Pectinogen		Pectinogen
14. Carre and Haynes (16)	Pectic acid	Ca Pectate	Pectin	
15. Farnell (17)			Pectinogen	
16. Wichmann (18) and Chernoff	Pectic acid			
17. Sucharipa (19)	Pectic acid		Protopectin Free Pectin Hydrolysis pectin†	Protopectin

* The fraction soluble in water.

† Pectin obtained on treatment with mild hydrolytic agents.

The term pectinic acid (20), designating pectic compounds of methoxyl content varying between that of pectic acid and completely methoxylated pectin, is also met with.

The A. C. S. nomenclature has been accepted both here and abroad, with the exception of the German and continental school of pectin chemists who tend to follow Ehrlich's terminology. Briefly stated, the latter is based on his own scheme of pectin hydrolysis. According to this, pectin, the material in the plant, yields hydrato-pectin (hydro pectin, pectin hydrate) on mild hydrolysis. Further action of hydrolytic agents yields the isomeric tetragalacturonic acids (pectolic and pectolactonic acids). These acids break down further into d-galacturonic acid.

Recently there has been described a series of pectin derivatives, nitro-pectin (21), acetyl-pectin, and formyl-pectin (22). In these, terminology analogous to that used for cellulose derivatives has been employed. This is justified on the basis of the similarity between cellulose and pectin derivatives.

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Chemical Properties of Pectic Compounds

The pectic substances are complex carbohydrates differing from the true carbohydrates by their acidic properties. Aldehyde reactions, for example reduction of Fehling's solution, are given only after the pectic compound has been hydrolyzed. Acid reactions such as ester and salt formation are shown by the material in the natural state where it is found as the methyl ester of a calcium or magnesium salt. As a hydroxylated acid it forms inner anhydrides, lactones, and enters into glucosidic combination, etc. Buston and Nanji report (1) that the methyl ester of pectic acid can be prepared by treating silver pectate with methyl iodide. The compound formed has properties resembling the original pectin (jellying power, etc.). The ethyl ester was found to be unstable. Pectic acid forms soluble alkali metal and ammonium salts, and insoluble salts of the alkaline earth and heavy metals. Characteristic compounds are formed with alkaloids (2). Ehrlich and Schubert report the cinchonine, brucine, and morphine compounds, with m. p. 178° C., 180° C., and 162° C., respectively.

Pectin is similar in properties to many of the other polysaccharides. Its similarity to the hemi-celluloses has been pointed out by O'Dwyer (3), Norris and Preece (4), and Norman (5). Norman and Norris (6) found that oxidation with Fenton's reagent (H_2O_2-ferrous acetate) caused de-carboxylation of the pectic acid with the formation of hemi-cellulose like derivatives. The similarity of pectin to the gums and mucins has been shown by Norman (7), Butler and Krechter (8), Hilger (9) and Neville (10). Pectin is similar to cellulose in that it has the same chain-like structure and forms nitro and acetyl derivates (11, 12) that are similar to the corresponding nitro- and acetyl-celluloses.

The hydrolytic products of pectin are covered in the discussion of the constitution of pectin. However, only the products of the mild hydrolysis of pectin were presented. Hydrolysis of pectic compounds in strong acid causes decarboxylation with the formation of furfural and carbon dioxide. The furfural is derived from both the pentose and galacturonic acid portions. Alkaline hydrolysis breaks down the pectic molecule to galacturonic acid, arabinose, and galactose. The composition of pectin varies with the temperature of extraction.

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Constitution of Pectic Compounds

Most of the studies of the constitution of pectin have been made since 1917, when Ehrlich published his research on the composition of the pectic molecule. Contributions have been made by others, some supporting Ehrlich's views and others opposing them. Because there is disagreement concerning the constitution of pectin, and because at least an approximate understanding of its structure is desirable, the different views of researchers will be considered, correlated and summarized.

Ehrlich's (1) first pectin studies were made on sugar beet residue. By extracting with boiling water or water heated under pressure he obtained a solution of "hydrato-pectin." This pectin was separated into two components, one being soluble and the other precipitating. By adding alcohol, the soluble fraction was found to be laevo-rotatory araban, and by hydrolysis yielded 93% dextro-rotatory l-arabinose.

The other fraction was considered by Ehrlich to be the main constituent of pectin and is a dextro-rotatory calcium or magnesium compound (2). This is the alcohol-insoluble material obtained from fruit extracts and is commonly termed pectin.

This pectin does not contain pentoses, but it yields pentoses by decarboxylation of the galacturonic acid. Oxidation of pectin yields 50% of mucic acid. Hydrolysis of pectin yields galactose, methyl alcohol and d-galacturonic acid. Mild acid-hydrolysis yields a dextro-rotatory galactose-galacturonic acid. Treatment of pectin with alkali yields a tetra-basic acid containing four molecules of d-galacturonic acid. Ehrlich names this d-tetra-galacturonic acid and considers it to be the basic structural unit of the pectic molecule. From these reactions Ehrlich concludes that the pectic material of plants is a calcium or magnesium salt of a complex anhydro arabino-galactose-methyl-tetra galacturonic acid. From the fact that treatment of water extracts of sugar beet residues with alcohol always yielded 25-35% araban, and it was impossible to obtain more even by boiling the residue

with 70% alcohol, it was concluded (2) that the araban fraction is a loosely-held component of the original pectic molecule, and not a hydrolytic product resulting from extraction. The presence of an araban component of the pectic molecule is due to decarboxylation of tetra-galacturonic acid (4).

This portion yields methyl alcohol, when hydrolysed or fermented. The complete list of hydrolytic products found by these workers were galacturonic acid, acetic acid, galactose, arabinose, and methyl alcohol. The presence of acetic acid in pectin is interesting because the only other biological materials that have been found to yield acetic acid are lignin, some celluloses, chitin, and chondroitin sulphuric acid. This classes pectin with the cellulosic and hemi-cellulosic plant materials. Mild hydrolysis of tetra-galacturonic acid first yields di-galacturonic acid, then hydrated and anhydrous forms of galacturonic acid.

These researches led to the conclusion that pectin is composed of four molecules of galacturonic acid, 1 molecule of galactose, 1 molecule of arabinose, 3 molecules of acetic acid, and 2 molecules of methyl alcohol. The actual pectic compound was regarded as tri-acetyl-arabino-galacto-dimethoxy-tetra-galacturonic acid, $C_{37}H_{42}O_{37}$. The calculated molecular weight is 1170, while the experimental value determined from cryosopic measurement is 1370. These are in agreement only on the assumption that pectin compound is the decarbohydrate.

Ehrlich and Schubert (3) studied the hot-water-soluble incrustations of flax, employing the methods used in their beet pectin studies. The alcohol-soluble portion, instead of being pure araban, was found to be a hexo-pentosan containing 55% pentoses, 17% d-galactose, 20% fructose, and probably some l-xylose. The composition corresponded to $C_{22}H_{37}O_{23}$. The alcohol insoluble portion yielded, by acid hydrolysis, di-galacturonic acid, di-galactose, l-arabinose, l-xylose, acetic acid, and methyl alcohol. Its elementary composition was found to be $C_{45}H_{68}O_{40}$. A resin-acid, somewhat similar to lignin, was found in the alcohol-insoluble fraction. This work substantiates Ehrlich's previous research on the constitution of pectin to a certain extent and presents a further complication in the presence of a xylose component.

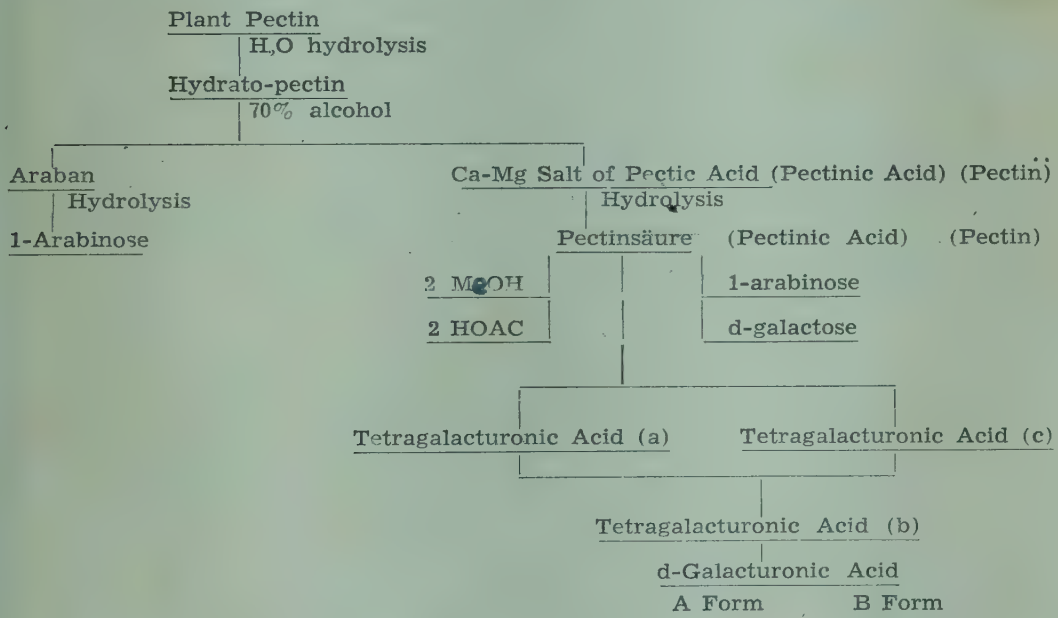
The pectin of orange peels (5) was found to be similar to that from beets. Hydrolysis yielded four molecules of galacturonic acid, and one molecule of galactose, one molecule of arabinose, two molecules of acetic acid, and two molecules of methyl alcohol. The empirical formula of $C_{41}H_{60}O_{36}$ is ascribed, nine molecules of water being eliminated on combination. This formula agrees with analyses and molecular weight determinations of pure pectic acid. The tetragalacturonic acid of orange and sugar beet pectins have identical properties. This further supports the view that the fundamental unit of the pectic molecule is tetra-galacturonic acid. Studies on currant and strawberry pectins show that they are composed of araban calcium, and magnesium salts of pectic acid fractions. No methyl pentoses or acetone were found in any of these materials.

From the general behavior, and because the composition and molecular weight determinations substantiate the fomula $C_{11}H_{16}O_{35}$, Ehrlich and Schubert (6) conclude that pectin is a dimethyl ester of a conjugated tetragalacturonic acid. The four molecules of galacturonic acid combine through their aldehyde groups in 1:4 linkage. In this combination are also found one molecule of arabinose and one of galactose with two acetyl groups attached to the chain.

Further study of tetragalacturonic acid indicated that it exists in three metameric forms. It was proven that these were (a) tetra-anhydro-tetragalacturonic acid (b) tri-anhydro-tetragalacturonic acid monolactone, and (c) hydrato tetra-anhydro tetragalacturonic acid. Each of these acids is formed by conjunction of hexose or hexuronic molecules by 1:4 linkage. The aldehyde group is masked and the carboxyl group is free or is in combination with metals, methyl groups, or possibly cellulose. Acid (a) is a closed-ring compound formed by the condensation of four molecules of galacturonic acid. Each of the four components possesses a 1:5 inner anhydride structure. Acid (c) contains one molecule of water more than (a). From the fact that this molecule of water is more firmly bound than water of hydration, it was concluded that it is taken up in hydration of an inner anhydride group. Acid (b) is an open-chain acid derived by splitting one molecule of water from acid (a) or two molecules of water from acid (b). The terminal carboxyl group of acid (b) is in lactone combination. Hydrolysis of these acids yields the two isomeric d-galacturonic acids.

It was found that certain fungi produce enzymes that quickly open the cyclic tetragalacturonic acids (a) and (c) to form (b). Longer action gives quantitative yields of d-galacturonic acid. Certain molds that decompose pectin in nature exhibit high activity on the pure tetragalacturonic acids; this is further evidence that the structural unit of the pectic molecule is tetra-galacturonic acid.

Ehrlich's work on pectin, its derivatives, and their relationship is summarized in the following manner:



Von Fellenberg's analysis (8,9,10) of beet pectic acid showed the molecule contains eight units of galacturonic acid, two of galactose, one of arabinose, and one of methyl pentose. He proved that the methyl alcohol originates from methyl ester groups. The methyl groups are combined with galacturonic acid; the fully esterified compound contains eight esterified groups. These groups can be removed successively to form "pectinic acids" of varying stages of de-esterification from the octamethylated pectin, to the

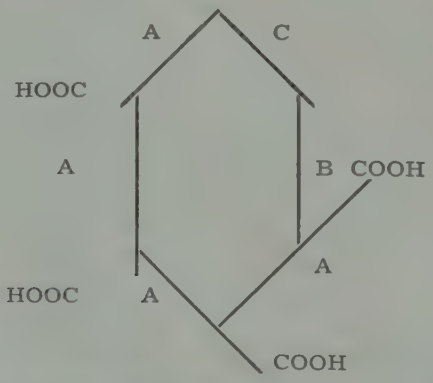
completely demethylated pectic acid. Theoretical values for methoxyl content of these pectinic acids on the basis of $C_{78}H_{120}O_{68}$, and experimental data of certain pectins are as follows:

Calc. for Octamethoxy pectin	11.94 per cent
Orange pectin	11.60 per cent
Calc. for Heptamethoxy pectin	10.50 per cent
Apple pectin	10.57 per cent
Calc. for Hexamethoxy pectin	9.07 per cent
Currant pectin	9.20 per cent
Calc. for Pentamethoxy pectin	7.61 per cent
Orange pectin	7.46 per cent

Von Fellenberg believes that the insoluble pectic compound of plant cell walls is a pectin-cellulose combination. The pectin cannot be removed by cold water, but can be by hot water or dilute acids. This view has been confirmed by Sucharipa (11) who claims to have dissolved the cellulose from purified lemon albedo with Schweitzer's reagent, leaving the pectin-cellulose compound unchanged. The insoluble material is considered to be proto-pectin. When treated with hydrolyzing agents, the protopectin yields pectin and cellulose in varying proportions. Each successive hydrolysis of the protopectin fraction shows fewer carboxyl radicals combined with methyl alcohol groups and more with cellulose than the fraction preceding. This indicates that protopectin is composed of a series of pectin-cellulose compounds of varying composition formed by condensation of cellulose with pectin.

Nanji, Paton, and Ling (12) support von Fellenberg's opinion on the origin of methyl from pectin but believe no methyl pentose is present in the pectic molecule. The work of Norris and Schryver (13) supports this view because they found only 0.15-3.4 per cent of methyl pentose in various pectinic acids. Calculated on the basis of the formula assigned to pectin by von Fellenberg, the methyl pentose content would be 14.6 per cent. Evidently the quantity of methyl pentose found is much smaller than would be expected, and may be merely an impurity.

Nanji, Paton, and Ling conclude that pectic acid is made up of four molecules of galacturonic acid, one of galactose, and one of arabinose, combined in the following manner



This structural formula shows the four galacturonic acid, the arabinose, and the galactose units in a six membered ring. This differs from Ehrlich's structural formula for pectic acid in that combination is by 1:5 instead of 1:4 glucosidic linkage, the ring contains galactose and arabinose, and the sugars and sugar acids are not given in pyranose and furanose structures.

Nanji, Paton, and Ling consider protopectin to be a Ca, Mg. or Fe salt, luble in dilute acids but not in water. Most of their analyses show that ıly two or three of the four carboxyl radicals are methylated. For this eason it is supposed that the remaining carboxyls are combined with metals. ın this manner several pectin molecules could be joined together to form a chain. Protopectin is considered to be the insoluble metal salts of pectic acid. These authors decline to recognize the existance of pectin, believing that protopectin and pectic acid are the only pectic compounds. Pectin is an intermediate mixture of a series between protopectin and pectic acid.

Henderson (14) doubts that arabinose is present in the pectic molecule. His researches show that pectic acid, prepared by ordinary methods, contains occluded sugars. The presence of arabinose as an impurity is held by him to account for the statements of previous workers that the pectic molecule contains arabinose. By precipitating pectic acid as the copper salt he obtained a compound that corresponds to galactose-tetragalacturonic acid,

Myers and Baker (15) found the basal unit of the pectic molecule is octagalacturonic acid, instead of tetra-galacturonic acid postulated by Ehrlich (2). However, they suggest that the eight-membered unit may be formed by condensation of two of Ehrlich's tetra-galacturonic acid molecules. As the four-membered tetra-galacturonic acid ring is formed by the condensation of four galacturonic acid molecules, with the elimination of four molecules of water, the eight-membered unit, consisting of two of these four-membered rings, may be formed by elimination of nine molecules of water. According to this, pectin is monarabino-monogalacto-diacetyl-hepta methoxylocto galacturonic acid, $C_{70}H_{98}O_{58}$. Fully methylated pectin possesses seven methyl ester groups and one free carboxyl. This free carboxyl is presumably combined with cellulose or metals in the plant tissue in the form of protopectin.

Bauer and Link, as well as Myer and Baker, (16) arrived at the same conclusions concerning the number of galacturonic acid molecules in the basal pectic unit. The former do not agree, however, to the four-membered ring structure proposed by Ehrlich or to the idea of Myers and Baker that the eight-membered pectic unit is composed of two of Ehrlich's four galacturonic acid rings. Their data indicate that pectin is essentially a poly-galacturonic acid combined in 1:4 glucosidic linkage. The value for **n** was found to vary beween six and eight. Pectin is the methyl compound, while protopectin could be an insoluble combination of metals or cellulose. A methyl alcohol-HCl method of hydrolysis is used in this research which is claimed to eliminate the degradation that occurs when hydrolysing pectin in aqueous solutions.

In a later work (17) Bauer and Link employed their methods of investigation on Ehrlich's tetragalacturonic acids. They found that Ehrlich's four-membered rings were constituted as stated above. They point out further that, whereas they consider that pectin contains 8-10 members, this is in reality the minimum size of the pectic molecule, the true length being indeterminable because of lack of knowledge of the degradation caused by the method of treatment.

SUMMARY

A review of researches on the constitution of pectin shows few facts i which all are in agreement but many for which there is little or no expla nation. It is certain that galacturonic acid is the main constituent of pecti substances. Associated with it are found araban, galactose, arabinose, aceti acid, methyl alcohol, and various metals such as Ca, Mg, and Fe. It i doubtful if methyl pentose or xylose are present.

The manner in which these constituents are combined is uncertain However, it is probable that the galacturonic acid molecules are combine with each other in typical 1:4 glucosidic linkage, the methyl alcohol as th methyl esters of the poly-galacturonic acid, and the metals as salts. Th manner of combination of the other components is unknown.

The structural formula proposed by Nanji, Paton, and Ling is inadequat for the reason that it does not explain the variations in arabinose and galac tose content found in many cases. The structural formulæ of Ehrlich, an Bauer and Link show the manner in which the galacturonic acid molecule are combined but are not complete for the reason that they do not indicat the true size of the molecule, or explain the manner of combinations wit arabinose, galactose, araban, etc.

X-ray studies of Meyer and Mark (18) and Van Iterson (19), and molec ular weight studies of Henglein and Fritschi (20) and Henglein and Schnei der (21) show that the minimum molecular weight of pectin is betwee 20,000 and 100,000. Marked similarity in structure to cellulose was noted This leads to the conclusion that the pectic molecule is essentially a lon chain poly-galacturonic acid molecule.

The origin and combination of galactose, arabinose, and araban in th pectic molecule must still be explained. A comparison of the structura formulæ of these compounds with those of galacturonic acid and poly-galac turonic acid indicates a possibility of formation of galactan, poly-galactu ronic acids, araban, and arabinose from galactose by a series of condensation decarboxylation, and hydrolytic reactions. From this the essential processe of synthesis and analysis of pectin in the plant can be indicated as follows

Galactose
| + Conjugation
Galactan
| + (Oxidation)
Poly-galacturonic acid
| + (de-carboxylation)
Araban
| + (hydrolysis)
l-arabinose

The presence of galactose in the pectic molecule can be explained on th basis of incomplete oxidation of the conjugated galactose chain (galactan) leaving galactose molecules at intervals. The presence of arabinose in th pectic chain is due to decarboxylation of galacturonic acid units. Araban is formed by decarboxylation of a large number of such units or of the entire pectic molecules. It is known that natural conditions found in the plant favor decarboxylation and hydrolysis.

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PHYSICAL PROPERTIES OF PECTIC COMPOUNDS

Pectin is a white amorphous solid having a specific gravity (1) of 1.180 at 20° C. When heated at 156° C. it turns brown and at 158° C. it decomposes with evolution of gas. In its natural state, pectin is isotropic, although it is possible to obtain a certain degree of orientation by drying it under strain. This is especially true in the case of calcium pectate, in which the calcium seems to facilitate the parallel orientation of pectin chains, possibly by combining with free carboxyl groups of adjacent micellæ.

Protopectin is difficultly soluble in cold water but dissolves when heated owing to partial hydrolysis. Pectin absorbs about 15 per cent of water without undergoing any change in physical appearance (2). When placed in cold water it swells and dissolves slowly. Its solution is hastened by heating, agitation, or addition of peptizing agents. Contrary to frequent statements, it has been shown (3,4) that pectic acid also is soluble in water, in the absence of calcium and magnesium ions. The alkali salts of pectic acid are very soluble.

Pectin can be precipitated from its solutions by the addition of miscible organic liquids. The concentration of alcohol necessary to cause precipitation depends somewhat upon the pectin concentration; high concentrations of pectin may be precipitated by one part of alcohol while several parts of alcohol are necessary to precipitate dilute concentrations of pectin. Also in very dilute pectin solutions, precipitation occurs only when traces of electrolyte are present. The physical form of the pectin precipitate is varied by different pectin concentrations. At low concentrations, the pectin precipitate is light and flocculent; at concentrations above 1 per cent, it is colloidal and jelly-like. According to Taft (5) pectin easily forms 2 per cent solutions in liquid ammonia.

Equivalent and Molecular Weights

Bonner (6) determined the equivalent weight of pectic acid by the cataphoretic method of De Jong (7) and found it to be 203. Estimated from the sodium content of the corresponding sodium pectate, the value was 229. The cataphoretic measurement is really the equivalent weight per unit charge, but since the seat of the electrical charge on the pectin-micelle is the carboxyl group, the measurement is one of equivalent weight. The pectic acid used in this determination was obtained from totally de-methylated pectin. In a similar manner, the equivalent weight of a pectinic acid obtained by acid-extraction was found to be 570. The corresponding value determined from the sodium salt prepared by treatment with a concentrated sodium chloride solution was 603.

The work of Ahmann (8) shows that the equivalent weight of pectic acid, determined from the sodium salt, is dependent on the conditions of hydrolysis. Weak hydrolysis does not completely demethylate the pectin and strong hydrolysis causes a rupture of the pectic acid nucleus. After working out the conditions of hydrolysis for preparing pure pectic acid, Ahmann prepared the sodium salt and determined its equivalent weight. This he found to be equal to 195. The equivalent weight of pectin, calculated from this value for pectic acid, is 209. Ahmann and Bonner assume that the equivalents of pectic acid and pectin differ by one molecule of methyl alcohol. The calculations show fair agreement with each other, and also with the equivalent weight of 208 calculated for the hypothetical pectic unit, methyl, galactose, tetragalacturonic acid.

The pectic molecule has a large and variable molecular weight and is in all probability a long chain composed of many units. Ehrlich and Schubert (9) concluded that pectic acid is a definite chemical compound. They determined the molecular weight by freezing-point methods and found it to be about 1300. Henglein and Schneider (10) state that Ehrlich's data is inconclusive because molecular weights of hydrophilic substances, such as pectin, cannot be determined by freezing-point, boiling-point, osmotic pressure, viscometric or centrifugal methods in polar solvents. In order to obtain compounds that might be studied in non-polar organic solvents, they prepared nitro-pectin by nitrating "hydratopectin." This compound is soluble in acetone, and has a molecular weight of 20,000, as determined by viscometric method of Staudinger. The molecular weight of the micellar unit, that is the equivalent weight, was 205. The equivalent weights of the nitro-pectic compounds corresponded to dinitro galactose-galacturonic acid. Thus, they conclude the pectin molecule is probably a chain-composed to 100-250 of galactose-galacturonic acid-units.

In a later work Schneider and Fritschi (11) studied acetyl- and formyl-pectin. Evidences of degradation of the pectic molecule, due to the methods of formation of these compounds, were encountered, especially when the treatment was prolonged. As in the case of nitro-pectin, solutions of the compounds in organic solvents were used for osmotic pressure and viscometric molecular weight determinations. It was found that the molecular weight of nitro-pectic acid was 20,000-40,000; nitrated hydrato-pectin was 30,000-50,000; nitro-pectin prepared directly from beet residue, 50,000-100,000; and acetyl pectin from the preceding nitro-pectin 30,000-100,000.

The filament nature of the pectin molecule, first established by X-ray studies (12,13), were confirmed by comparison of the specific viscosity-mole-

cular weight ratio of a series of nitro-pectins, with the same values for cellulose and starch. These studies showed the pectin molecule to be of the same nature as cellulose, only not quite as long. The conclusion is drawn, that the true molecular weight of pectin is at least as great as those found for the nitro, acetyl, and formyl derivatives, and perhaps greater, as such treatment could, if anything, cause only degradation of the original molecule.

The length of pectic filaments was shown by Mehlitz (14) to be of the magnitude of 10^{-5} centimeters. Measurements, made by filtration of pectic solutions through membranes of graded pore-size, showed that the pectic molecules were 10 per cent smaller than 0.2 microns, 60 per cent between 0.2 and 0.6 microns, and 30 per cent greater than 0.6 microns. It was also observed that decrease in pore-size was accompanied by decrease in total acidity, hydrogen ion concentration, and viscosity of the dialyzate.

Viscosity of Pectic Solutions

The relative viscosity of pectic solutions increases with the concentration of pectin. This relationship is not a simple one, as the degree of polymerization of the pectin-molecule, the number of additional radicles combined with the micellar unit, hydrophilic effects and, the presence of cations or organic materials, may cause variations in viscosity, independent of the concentrations. Because of these modifying influences it is impossible to correlate definitely viscosity with concentration.

It is considered (15) that the jellying power increases with the degree of polymerization of the pectin-molecule and that it is independent of the number of radicals or groups such as methoxyl, acetyl, etc., that may be combined with the pectic chain. The viscosity, on the other hand, is a function of the molecular weight of the entire pectin-molecule and consequently varies both with the number of constituents on the chain and with the degree of polymerization. For this reason it is impossible to correlate viscosity-measurements with jellying power. Changes, involving the loss of methoxyl groups but not depolymerization, would result in a drop in viscosity but not in jellying power. Changes, involving only de-polymerization of the pectin molecule, should correlate with viscosity.

The viscosity of pectin solutions varies with the number and kind of ions in the solution. Gluckmann (16) treated a 10 per cent solution with the chlorides of Al, Ba Ca, K. Na. After 24 hours the pectin was precipitated with alcohol and the specific viscosities were determined. It was found "that the ratio of the viscosity of an electrolyte solution to that of water was equal to the ratio of the viscosity of a pectin solution containing the electrolyte to that of the pure pectin solution." In each case the specific ion-effects were those of the cations, because no appreciable absorption of the chloride ion was noted.

The increase in viscosity of pectin-solutions, due to the presence of organic substances, was shown by Glückmann (17) to increase with the series, organic acids, polyhydric alcohol, monohydric alcohols and ketones, and sucrose.

The changes in viscosity of pectin solutions caused by the presence of other substances is not due to variations in the degree of polymerization or to the constitution of the pectic molecule. This was evidenced by the fact that there was no accompanying change in the micellar dimensions as shown

by the ultramicroscopic picture. Since the above organic series has the same order as Kurbatow's association-series, it is concluded that foreign substances increase the viscosity of pectin solutions by reducing the association of the water.

Index of Refraction of Pectic Solutions

Refractive indices (18) of solutions of hydrato-pectin, obtained by extracting dried apples and beets with boiling water, were determined. It was found that the ratio of the difference between the index of refraction of the pectin solutions and the water to the concentration of the pectin was a constant. The relationship was studied with solutions containing the various cations, sodium, calcium, barium, and hydrogen. It was found that sodium ions produced an increase in the constant, calcium no change, and barium and hydrogen a decrease. The relationship between the indices of refraction and concentration of pectin solutions is linear, in the absence of specific ion effects.

Optical Rotation of Pectin Solutions

The optical properties of pectic compounds vary widely. However, all of the components except araban are strongly dextro-rotatory. Variations in rotation are dependent on the conditions of extraction, age of solution, etc. Specific rotations of pectic substances are given in the following table:

COMPOUND	$[\alpha]_D^{20}$	Reference
PECTIN		
Sugar beet (water extract)	+100°-160°	(19)
Sugar beet (treated with HCl)	+220°	(19)
PECTIC ACID		
Lemon peel	+183°	(20)
Lemon (technical)	+240°	(21)
Orange (from easily soluble pectin)	+189.7°	(11)
Orange (from difficultly soluble pectin)	+175.3°	(11)
Sugar beet (from easily soluble pectin)	+197.50°	(11)
Sugar beet (from difficultly soluble pectin)	+132.10°	(11)
Flax	+119.7°	(11)
TETRA GALACTURONIC ACID		
(a)	+275°	(19)
(b)	+250°	(11)
(c)	+285°	(11)
d-GALACTURONIC ACID		
A } after standing 24 hours	+50.9°	(19)
B } after standing 24 hours	+55.3°	(19)
ARABAN	-123° to -176°	(19)

Colloidal Properties

Pectin is a reversible, hydrophilic colloid. It forms opalescent solutions that exhibit the Tyndall effect and typical colloidal behavior under the ultramicroscope (22). Pectin from apples, quinces, and beets are precipitated by copper sulphate, lead nitrate, basic and neutral lead acetate. The salts $AgNO_3$, $HgCl_2$, $Cd(NO_3)_2$, $NiSO_4$, $FeSO_4$, $CdCl_2$, $ZuSO_4$, $MnCl_2$, $SrCl_2$, $BaCl_2$, or the alkali salts do not precipitate the pectin from any of these sources (22).

Von Fellenberg concludes that precipitation by heavy metal salts is caused by oppositely charged metal-ions, rather than by the formation of insoluble metal-salts of pectih. This is evidenced by the fact that pectic precipitation is independent of the concentration of the metallic ion. When a threshold-concentration of the metal-ion is reached the entire mass of pectin

precipitates. Depending on the pectic concentration this precipitate may be flocculent, or a rigid gel. As shown by the ultra-microscope picture (23), the pectin remains unchanged, and can be obtained from the metal-pectin combination by washing out the metal with acid solution.

Flocculation by electrolytes or oppositely charged colloids occurs only when both the electro-negative and hydrophilic properties of pectin are reduced. Pectin is weakly electro-negative and strongly hydrophilic and is not easily precipitated by cations because of difficulty in overcoming the affinity of the pectin-micelle for water. Pectic acid, on the other hand, is strongly electro-negative and weakly hydrophilic, and is precipitated by slight traces of cations. The pectinic acids are intermediate between pectin and pectic acid in their precipitability by electrolytes. The affinity of pectinic acids for water and the strength of their micellar charge varies with the number of free carboxyl groups in the molecule. The hydrophilic properties decrease and the electro-negativity increases, with increase in number of carboxyl groups.

Pectin is also precipitated by other materials that reduce its association with water, such as alcohol, acetone, ammonium sulphate, and sugar solutions.

Pectin Gels

Pectic solutions form stable gels under certain conditions. In general, these conditions decrease the solubility of pectin to the point that it becomes the continuous phase. The most common example of this is the pectin-sugar jelly. Pectic gels, however, may be formed from solutions of heavy metal salts and by the action of organic liquids.

The pectin-sugar jelly is the most important of the pectic gels and will be considered in detail. Tarr (24) found that the nature of the acid used in jelly formation was important, but that the pH was also important. No gelation occurred above a pH of 3.46, and acid-concentrations below the pH of 3.1 caused synerisis. These tests were made containing solutions 67 per cent of sugar and 0.8 per cent of apple-pectin. Tarr found it impossible to prepare gels that contained less than 64 per cent sugar, and this same concentration of pectin.

Ohn (25) substantiated the importance of pH rather than total acidity in jelly-formation. Using 0.4 per cent citrus pectin-solutions, she found that the best gels had a sugar-content of 62.5 per cent and a pH of 2.60. She further prepared jellies that contained as low as 55 per cent of sugar. Dore (26) states that, for the sugar-pectin ratio of 65: 1, the optimum pH is 3.37. Lowering the pH to 3.1 allowed gels with sugar: pectin ratios as high as 90.1 to be prepared.

Baker (27) studied the factors that influence the strength of jellies. He tested the effect of sulfuric, tartaric, and citric acids. The optimum pH values for gel-formation were different for each, varying from 3.05 to 3.30. Sulfuric acid gave 25 per cent stronger jellies than those made with citric acid. Tartaric acid was intermediate between sulfuric and citric acids in jelly forming power. In each case, increase in acidity beyond the optimum yielded weak jellies due to synerisis; decreased acidity reduced the jelly to a sticky syrup. In studying the effect of sugar concentration on jellies, it was found that only sugar concentrations within 61-75 per cent gave jellies of measurable strength. In each case the jellies were prepared by mixing 100 grams of sugar, one gram of pectin and 120 cc. of tartaric acid solution

containing .001 equivalents of acid, this mixture was boiled down to the desired weight. Maximum jelly strength was given by the gel with 69 per cent of sugar. This value for the optimum sugar-concentration varies with time of cooking and pH. The time of cooking in these experiments was fairly long and was not a constant factor throughout. For this reason the jelly-power would vary because of variation of the sucrose-glucose ratio. For this and other reasons an optimum value of 69 per cent sugar is somewhat erroneous. Doubling the concentration of pectin permitted 15 per cent more water being added to the final jelly. The jellies formed were more crisp in texture, but somewhat cloudy.

The effect of heat on the jelling strength of pectin was determined for pectin-acid solutions, with and without added sugar. When the pectin was boiled without sugar the jelling power was reduced 37 per cent in twelve minutes, the reduction being a linear function of the time. When the pectin was boiled after the sugar was added the jelling power of the pectin was not reduced but was increased somewhat. This increase in jelling power may be correlated with the increase in invert-sugar due to boiling.

The effect of temperature on the strength of jellies was studied over the range of 2°-30° C. The strength was found to be dependent on the temperature. An increase of twenty degrees from 10°-30° C. caused a 30 per cent increase in the jelling power. The strength of jellies was also found to increase linearly with the age of the jelly.

Pectic gels are formed when heavy metal salts such as copper sulphate, lead nitrate and basic or neutral lead acetate (28) are added to pectic solutions. Glückmann (29) has shown that organic liquids cause pectic solutions to gel. The gelling power increases in the order of organic acids, polyatomic alcohols, monatomic alcohols and ketones, and sucrose. Gelling power also increases with the length of the carbon chains in homologous series. Glückmann (30) also studied the effect of metal-ions on the strength of pectin-alcohols gels. With sodium, potassium, lithium, or magnesium ion in the pectic solution he found that more alcohol was required to precipitate the pectin. Calcium ions had no effect; aluminum and barium ions reduced the quantity of alcohol necessary to cause precipitation. In each case the cation was fixed in the pectin molecule and could not be removed by washing. These cations aid in the gelation in the same manner as they cause coagulation of irreversible colloids.

Gaponenkov and Muimrikova (31) tested the effect of salts on pectin-sugar gels. They found that potassium and sodium chlorides decrease the gel-strength; magnesium chloride has no effect; calcium, aluminum and barium chlorides increase the gel-strength. Halliday and Bailey (32) report that 0.5-1.0 per cent of $CaCl_2$ increases the strength of pectin-sugar gels. Gaponenkov (33) showed that pectin-sugar gels could be prepared by substituting alcohol for the acid. Thus better gels were obtained. However, they were unstable and crystallized within a week.

Either sucrose or glucose are suitable (34) for pectin-sugar-acid jellies as long as the concentrations are correct. Fiedler (35) states that inversion of cane sugar by boiling may prevent jelly formation, though the jelling power of the pectin remains undestroyed.

Pectin is not altered chemically in forming a jelly; its only change is one of state. Sucharipa (36) showed that pectin separated from a pectin-sugar jelly retained its original characteristics and jelling power.

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PHYSIOLOGICAL PROPERTIES OF PECTIN

There is little knowledge concerning the fate of pectin in animal digestion. Althausen and Weyer (1) found no appreciable increase in blood sugar in normal subjects, upon the addition of galactose, one of the hydrolytic products of pectin. Imhäuser (2) administered pectin orally to dogs and found no increase in blood sugar. Neither was fat deposition in the liver prevented by pectin in phloridzinized dogs. However, there was a marked anti-ketogenic effect, indicating utilization of at least part of the pectic material.

Pectin has been found to be an efficient hemostat (3,4,5). Rieser (6) has found that peroral, intravenous, or subcutaneous injection of pectin into rabbits reduces the average blood clotting time from 40 to 50 per cent. The clotting action is believed to be due to an indirect action of pectin in which the active clotting agent results from the contact of pectin with the endothelium of the blood. In a series of similar experiments (7) it was found that the blood-clotting effect occurred within three hours after subcutaneous or peroral injection and lasted two hours. Clotting occurs instantly after intravenous injection and lasts many hours. Thrombosis or embolisms were

never seen and the toxicity is very low, as much as 100 cubic centimeters of 2.5 per cent pectin solution being well borne.

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Chapter VI

PECTIC ENZYMES

In plant tissues there are a number of enzymes or ferments that play very important roles in the natural processes of growth, ripening, overripening and ultimate decay. In the commercial separation of pectin from plant tissues, in the storage of fruits, in fruit juices, in wines, or in the manufacture of jams, jellies, and marmalades, these enzymes exercise influences that may lead to spoilage.

The several enzymes that act on pectic materials are not known from the standpoint of their chemical composition. Classification on the basis of their reaction products is also difficult because of obscurity of the chemistry involved. Three of these enzymes are commonly recognized and are defined as follows by the Committee on Nomenclature of Pectin of the Agriculture—Food Division of the American Chemical Society (1).

Protopectinase

"The term applied to the enzyme which hydrolyzes or dissolves protopectin with the resultant separation of the plant cells from each other, usually spoken of as maceration. Presumably the product of this hydrolysis is pectin. The term protopectinase supersedes the older term pectosinase with which it is synonymous."

Pectinase

"The term applied to the enzyme which hydrolyzes pectin and pectic acid into their simplest cleavage products, which are probably arabinase, galactase and galacturonic acid."

Pectase

"The term applied to the enzyme which converts pectin into pectic acid, the latter becoming a gel, especially in the presence of calcium (or barium or strontium) salts."

Evidences of existence of these three enzymes was summarized by Willaman and Davison (2) in the following: All three are not always found in the same plant material; they reveal different temperatures of inactivation and optimum pH; they can be separated more or less completely by alcohol precipitation methods; only protopectinase is appreciably absorbed by filter paper; only pectase hydrolyzes ester linkages.

Two other enzymes have been suggested by Ehrlich (3)—pectolase and arabanase. These attack respectively the tetra-galacturonic acid and the araban components of the pectic molecule.

Industrial Importance of Pectic Enzymes

The process of retting of flax, ramie, and bast fibers has long been of great industrial importance. The harvested stalks are allowed to lie in a damp place or in water for a period of time, and the binding material decomposes leaving the free fibers. It is known that the decomposition involves the destruction of the protopectin by pectic enzymes, produced by certain bacteria normally found growing on the stalks and in the soil.

Similarly, the natural decomposition of the slimy coating of the coffee bean, the destructive action of molds and bacteria on fruit and vegetables, the clarification of fruit juices on standing, and the natural spoilage of fruits, are recognized now as due to pectic enzymes.

A recently developed technical application of these enzymes is as an aid to the clarification of wines and fruit juices. In many cases the sedimentation and turbidity of these products is due to the slow change of soluble pectin to insoluble pectic acid. The usual process is to let stand until natural defecation is complete. This is time consuming; the process can be hastened by treatment of the juice with pectase. After a short time the pectic material precipitates and can be filtered off. If pectinase is used instead of pectase, the pectin is hydrolyzed to stable, soluble sugars and these do not form precipitates on further standing. The latter process has the further advantage of retaining the products of hydrolysis. Because the bouquet and flavor of wines and juices are somewhat due to these products (4), the pectinase treatment is preferable.

At present there are two commercial pectinase preparations on the market, "Pectinol" (5) of the Rohm and Haas Company of Bristol, Pennsylvania, and "Filtragol" of the I. G. F. Farbinindustrie Aktiengesellschaft of Germany. Both of these are used in enzymatic clarification of fruit juices. One pound of "Pectinol" is claimed (6) to clarify 10 gallons of juice.

Pectic Changes Occurring During Ripening and Storage of Fruit

The changes taking place during the natural ripening of fruit and vegetables are interesting because a very striking agreement is found between the softening and the decrease in protopectin. A study of these changes in peaches (7) led to the conclusion that the transformation of insoluble protopectin to pectin was the only one that occurred. It was found that the sum of the protopectin and pectin was practically constant up to the overripe stage, at which point pectinase and pectase reactions also come into play. Furthermore, these investigators found that ripening and softening of fruits were simultaneous with pectic enzyme action. The rate of these changes is influenced by temperature, increasing enormously with a rise of a few degrees and reaching a maximum at about ten degrees below the temperature of deactivation of the enzymes. However, since heat otherwise modifies or damages the fruit, it is not commonly used to preserve fruit in the fresh condition. Preferably and practicably, the retardation of natural changes in fruit is controlled, by freezing or storage at moderately low temperature.

Carre (8) found, with Bramley's Seedling apples stored at 34° F. from October to August, that the protopectin and pectin remained constant for five months. The protopectin then decreased and for about three months this change was paralleled by an increase of pectin. After this the concentrations of the pectin decreased quite rapidly.

Emmett (9) studied pectic changes occurring in pears at certain low temperature. At 54° F. the fruit ripened in ten to twelve days and at 34° F. there was little indication of ripening even after 180 days. Also it was found that the rate of decomposition of the pectic substances was closely paralleled by the rate of ripening. He concluded that formation of soluble pectin is the chief factor concerned in ripening and softening and accounts wholly for the decrease in pectic substances, also lack of keeping qualities was due mainly to a rapid rate of degradation of the pectic compounds. It was also observed that breaking down of pectin in pears occurred more rapidly at 12° C., and more slowly at 1° C., than samples of apples studied.

This led to the conclusion that in low acid fruits the process is enzymatic while in high acid fruits acid hydrolysis is the important factor.

Similar observations and conclusions of pectic changes and ripening were made on tomatoes during their ripening on the vine (10).

Change of Jelling Power in Cold Storage and Frozen Fruit

Cold storage and frozen fruits retain their jellying power longer than fresh fruits do. The process of pectin decomposition is retarded but is not stopped even in frozen fruits. According to Morris (11) raspberries lose 15-25 per cent of their jellying power in four month's storage at -10° C.; gooseberries, about 35 per cent under the same conditions. At -20° C. only slight changes were observed. When the berries were heated before freezing the jellying power remained unchanged during storage.

General Consideration of Industrial Importance of Pectic Enzymes

It is seen that the action of pectic enzymes is always a factor in agriculture. This is especially true in processes where the material is macerated and allowed to stand some time before destroying the enzymes. This is due to the fact that maceration ruptures the cells and the liberated enzymes come into intimate contact with pectic cellular material. When cell walls are broken, increased destruction by enzymes is observable, for example, the rapid browning of pulped fruit.

In the manufacture of jelly and jam it is important that sliced or pulped fruit be treated immediately after pulping to destroy the enzymes. If heat is applied for this purpose, it must be continued only so long as is necessary to deactivate the enzymes. Further heating tends to modify the quality of the product as well as destroy the pectin.

Another possible method of inhibiting undesirable enzymatic action comprises adding the acid that becomes a part of the final jelly to the sliced fruit. This is somewhat objectionable because this acid tends to destroy pectin during the boiling process. Also early addition of the acid may cause premature setting of the jelly. Since the sugars occurring in fruit juices prevent the action of acids on pectin, it is possible that added sugar will prevent enzyme action. This may be a very simple remedy.

In mass production of canned fruit it is very important to make preliminary tests to ascertain the effects of enzymes. This can be done by destroying the enzymes by heating at 150° F. for ten minutes and then proceeding with the process. The product of this treatment is then compared in form, color and flavor with that obtained by the customary process.

Occurrence, Preparation, and Properties of the Pectic Enzymes

Protopectinase (pectosinase, pectosase, or propectinase)

Protopectinase occurs widely distributed in plant materials, especially in certain species of fungi and bacteria (44). It is also found in fruits and vegetables as apples (12), pears (13), peaches, and tomatoes (14).

The usual sources of protopectinases are the fungi; Bortrytis cinerea (15), various species of Rhizopus (16)—mainly Rhizopus tritici, Sclerotinia libertiana (17), Penecillium (sp) (18), and B. carotovorous (19).

The enzyme can be obtained either from the culture medium or by extracting it from the macerated mycelium with water. Harter and Weimer (20) were unable to obtain protopectinase from R. tritici grown on artificial

media, but found abundant production of it in the mycelium and medium when the organism was grown on vegetables or pectin.

Protopectinase extracts and solutions lose their activity quite rapidly. However, a very stable preparation can be obtained by precipitating the extract or centrifuging the media with acetone or alcohol (80 per cent), and drying the precipitate in vacuum. Filtration through paper materially reduces the activity of the enzyme (21).

Estimation of Protopectinase Activity

The relative strengths of preparations of this enzyme can be estimated by their action on plant tissues. Davison and Willaman (22) employed potato strips of definite dimensions. These were placed in the enzyme solution and weights were attached to the end of each. The relative activities are then judged by the length of time elapsing before the strips break.

Properties

Protopectinase, as the name implies, causes hydrolysis of the protopectin present in cell walls and the middle lamella of plant tissue. The product of this action is probably pectin, although it is difficult to prove this because subsequent changes are caused by pectinase and pectase action. Carre (23) states that there is a difference between enzymes that act upon the cell-wall protopectin and enzymes that act upon the middle lamella protopectin. She concludes that protopectinase causes solution only of protopectin in the cell-wall. However, this conception is not generally accepted.

If the insoluble pectin-material of the plant is considered to be composed of an araban and a Ca-Mg pectate portion, as stated by Ehrlich (24), it is possible that protopectinase splits this into the separate components.

Protopectinase was found by Willaman and Davison (25) to be completely absorbed upon kaolin, Norite, and Floyd reagent. By the usual methods these authors were unable to free the enzyme from these absorbing agents. The temperature of deactivation was found to be 48° C. for twenty minutes. The optimum pH is about 5; the optimum temperature is between 37° and 40° C. (26).

Pectinase

Pectinase was first discovered in malt (27). It is found widely distributed throughout the lower forms of plants (28). Pitman and Cruess (29) made systematic studies of its occurrence in bacteria, yeasts, and molds. They found the greatest activity of pectinase in P. glaucum (expansum) and Pythium (sp), with lesser amounts produced by Cytospora (sp), Bortrytis (sp), Penecillium (sp) and Rhizopus nigricans.

The yeasts, Sarcharomyces elligsoideus—Burgundy and Saccharomyces cerevisiæ, show little or no activity in reducing the jelly power of the pectin. However, the torulæ and mycodermæ show slight activity in this respect.

The bacteria studied, B. aceti and B. amylovorous, had little effect in solution of the pH of fruit. Other reported sources of pectinase are Taka-diastase (30), R. tritici (32), Sclerotinia cinerea, Bortrytis cinerea, the colon-typhoid group of bacteria (32), and the mucilaginous coating of coffee beans (28).

Preparation

The best commercial source of this enzyme is mold extract. The enzyme is precipitated by alcohol, or by saturated solutions of ammonium sulphate or phosphate. Menon (33) found that the enzyme activity was very definitely affected by the composition of the substrate. The use of pectin in the medium was found to promote pectinase production. Willaman (34) found that a preparation showing great activity could be prepared by macerating the mycelia and extracting with water. This extract was centrifuged and dialyzed through a colloidon membrane to remove ionic impurities. The solution was again centrifuged, made up to 50 per cent alcohol, centrifuged, the enzyme finally precipitated from the centrifugate at an alcohol concentration of 85 per cent and dried in vacuo.

Estimation of Pectinase Activity

Pectinase activity can be measured in various ways. Its quantitative estimation is based on the formation of reducing sugars, methyl alcohol, or any one of the other decomposition products of pectin. Owing to uncertainty of the chemistry of pectinase action, there is no definite means of correlating these changes with a specific enzyme. For this reason the only suitable method is based on the decrease in pectin content. The possibility of accompanying pectase action influencing the results must be taken into consideration. This is best detected by running parallel studies on the loss of jelling power and on the formation of pectic acid.

Pitman and Cruess (35) compared viscosity measurements with jelly power and pectin concentration to obtain a basis of estimating pectinase action in the presence of pectase.

Pectinase seems to be composed of several enzymes rather than a specific individual. The fact that the changes involved in the degradation of pectin to its simple components are many, makes it difficult to believe that a single enzyme is operative. Ehrlich (36) has isolated the enzyme that causes the hydrolysis of tetragalacturonic acid to d-galacturonic acid. This enzyme he calls pectolase. Until the other enzymes of this group are isolated and studied separately, the measurements of pectinase activity will lack significance.

Properties

The definition of pectinase itself indicates lack of knowledge of the chemistry of the changes involved. All that is known is that the process yields pentose and hexose sugars, uronic acids, and methyl alcohol. Thus pectinase seems to be a generic rather than a specific term.

This enzyme also decomposes pectic acid (37), and at a slower rate its calcium salts, in the same manner as it does pectin. The greatest change in the viscosity, occurring during pectin hydrolysis, takes place at the point of disruption of the pectic acid structure to a smaller molecule of galacturonic acid. Since the presence of calcium or other heavy metal cations cause an apparent difference of enzyme activity, the viscosity change is not a good method of measuring pectinase activity. Another possible source of error in estimating pectinase activity lies in the possibility of inhibiting substances being present. This has been found to be true in the case of media containing tannin, small quantities being sufficient to reduce the activity materially (38).

Since it is probable that the optimum conditions of the several enzymes composing the pectinase group are different, it is impossible to set any optimum of conditions. The optima might be definite for pectinase from one particular source, where the relative concentration of each component is constant, but for preparations from different sources large variations are to be expected.

Davison and Willaman (39) found that pH optima of their mold preparations to be about 3.0 and the temperature of deactivation at 60° C. Kertesz (40) found the corresponding pH value for Penecillium extracts to be 3.0, 3.5. The optimum temperature was found to be about 40° C. Waksman and Allen (41), Mehlitz and Scheur (42), and Mehlitz and Maas (43) report substantially the same data.

Pectolase and pectase can be considered members of the pectinase group, and have pH optima of 4.5-6.3, and increased activity with increasing pH, respectively. Comparisons of the optimum pH's given for the entire pectinase group indicate that the component having the greatest activity is still unknown.

Pectolase (tetragalacturonase) and pectase (pectin demethoxylase) are evidently included in the definition of pectinase and should therefore be considered members of this group.

PECTASE

Occurrence

Pectase occurs very widely disseminated throughout the plant kingdom, being commonly found in the members of the following Families (44).

FAMILY	GENUS	COMMON NAME	PORTION OF PLANT
Gramineæ	Zea Mays	Maize	Leaf
	Lolium perénne	Ray-grass	Leaf
Iridaceæ	Iris florentina	Florence flag	Leaf
Polygonaceæ	Rheum rhapónticum	Pie plant	Leaf
Chenapodiaceæ	Beta vulgaris	Common beet	In rhizome
		A sugar beet	
Saxifragaceæ	Ribres rubrum	Red currant	In juice of fruit
	Ribres nigrum	Black currant	In juice of fruit
	Ribres grossularia	Gooseberry	In juice of fruit
Roseaceæ	Pirus malus	Apple	In juice of fruit
	Pirus communis	Pear	In juice of fruit
Leguminosæ	Trifolium pratense	Red clover	In young leaves & shoots
	Medicago sativa	Lucerne (alfalfa)	In young leaves & seeds
Vitaceæ	Vitis vinifera	Grape	Nearly ripe fruit
Umbelliferæ	Daucus carota	Carrot	Rhizome
Oleaceæ	Syringa vulgaris	Common lilac	Leaves
Solanaceæ	Solanum tuberòsum	Potato	Leaves
	Nicotiana tobacum	Tobacco	Leaves
	Solanum lycopersicum	Tomato	Fruit
Rubiaceæ	Rubia tinctoria	Common madder	Rhizome

In addition to these sources a very active pectase preparation has been obtained from the fungi Sclerotinia cinerea (45), and Penecillium (sp) (46) grown on media containing pectin.

Preparation

Fremy (47), the discoverer of this enzyme, prepared it by alcoholic precipitation of plant juices. Davison and Willaman (48) obtained active preparations from clover juice and extracts of corn pollen. The juice was pressed out of the clover, and allowed to stand in the dark for 24 hours when natural defecation was complete. Chloroform was added as a preservative. After removing the precipitated material, the filtrate was evaporated at room temperature and dried over sulphuric acid. The corn pollen was extracted several hours with water, centrifuged, and the enzyme precipitated from the centrifugate with two volumes of alcohol.

Mehlitz (49) obtained a pectase preparation of very high activity by pressing the juice from lucerne and treating in the manner described above for clover, except that, instead of evaporating the filtrate it was treated with alcohol. The precipitate was dissolved in water, reprecipitated with alcohol, and dried over calcium chloride.

Paul and Grandseigne (50, 51) describe a process whereby readily soluble pectase preparations can be obtained by precipitating the enzyme with acetone in the presence of an added, soluble, colloidal material, such as gum tragacanth, starch, etc. It is then dried at 50° C. The same authors (52) describe a superior method for extracting the enzymes. The material is ground at 50° C. with 2-4 times its weight of water, allowed to macerate 36-48 hours, and is then filtered. Pectin and animal charcoal are used to purify this filtrate. The dried product is prepared from the purified extract by the method described above.

Estimation of Pectase Activity

Quantitative estimation of pectase activity must be based either on decrease in pectin or on increase in pectic acid or methyl alcohol. Many pectase studies are based on the estimation of the gel forming capacity of the enzyme, whereas it has been shown (56) that such gel-formation involves other than enzymatic processes. For this reason such data are of questionable value in estimating pectase activity. On the other hand, in the clarification of wines and fruit juices, methods based on the gel-forming capacity of the enzyme are important.

Properties

Pectase is defined (1) as the enzyme that converts pectin into pectic acid. Fremy (53) originally defined it by its capacity to yield a gel in pectin solutions. The latter conception is still held by some, notwithstanding the fact that it has been known for a long time (54) (55) that gel formation is a secondary reaction between pectic acid, the true product of pectase action, and calcium, barium, or other metallic ions present.

Kertesz (56) made a critical study of the mechanism of pectase action in calcium free media, and found that the reaction follows the monomolecular equation up to the point of 60 per cent of demethylation of the pectin. In no case did gel formation occur. Paralleling the demethylation study, the author also investigated the factors involved in calcium pectate gel formation. He found that the latter reaction was characteristic and its pH optimum varied with the percentage of demethylation of the pectin. This shows a dependence of gel formation upon the pectin-pectic acid ratio. There is no threshold value for the ratio, for it was found that gel formation occurred in slightly demethylated pectins under certain conditions and sometimes not until demethylation had proceeded to a much larger stage. It is evident

therefore that measurements of pectase activity based on gel formation are erroneous because they are measurements of the sum of two or more reactions.

That pectase is an esterase has been strikingly illustrated in several ways. Neuberg and Ostendorf (57) found methyl alcohol and calcium tartrate were formed when methyl d-tartrate was treated with pectase in the presence of calcium ions. Kertesz (58) obtained gels upon treating pectin solutions containing calcium with pancreatic lipase. He obtained the same effects by the use of a lipase extract from castor beans.

The same author (59) points out the similarity between the action of rennin and pectase. In the rennin coagulation of milk the process involves two stages, (1), that of the enzyme action splitting casein into a slightly smaller molecule paracasein, and (2), coagulation of the latter. The analogy is drawn between the two enzymes that both cause a slight degradation in the original molecule and in both cases the presence of calcium, strontium, or barium ions causes the formation of a precipitate or gel.

Kertesz (60) determined the pH optimum of pectase action and compared these with percentage of demethylation occurring under definite conditions. By subtracting the values given by a pectase-free solution under the same conditions, an activity curve showing a maximum at pH 6.5 was obtained

The temperature of thermal deactivation of pectase is 68°-70° C. for minutes (61). Light and air also have a deleterious effect on pectase activ according to Tserevitinov and Rozanova (62). Davison and Willaman (63 report that their pectase preparations did not change in activity while sto for four months while pollen grain preparations showed high activity storage for six to eight years.

Concentration of pectase solution by ultrafiltration was effected by Mc (64). With membranes of pore size from 2-0.2 microns, no concentrat was obtained. Using membranes with pore diameter of 0.2 microns and l he obtained a concentrate having an activity twenty times that of the origir The author concludes that this method is unsatisfactory for commer purposes.

Mehlitz also studied the effects on pectase activity of alcohol precipit tion, sugar concentration, and treatment with absorbent charcoal. He fou that precipitation by alcohol only affects the activity by increasing the coagulation time, the gel ultimately attaining the same hardness as when not treated. In the same manner, increased sugar concentration causes onl increase in coagulation time, and not in final strength. A 45 per cent sucro solution requires three times as long to coagulate as a sugar-free gel. Trea ment with decolorizing charcoal is without effect on the activity of t filtrate.

Pectase Gel Formation

Most of the reported data on pectase activity is based on its capacity form a gel. This phenomenon and the conditions influencing it are both theoretical and practical interest.

The optimum condition (65) of temperature and pH vary considerably. Von Euler and Svanberg (66) found the optimum pH is 4.3 for the members of the currant family. Davison and Willaman (67) failed to detect any optimum value for pectase preparations from Sclerotinia cinerea. Tserevitinov (68) found that enzyme optima for various fruit juices varied widely and even of juice from the same fruit. In the case of raspberries (69) the op-

num was 3.3, while for cider it was above 7.0, with corresponding tempera-re optima of 55°-60° and 60°-65°.

Mehlitz (70) made a detailed study of the optimum values of calcium, ctin, hydrogen ion, and pectase concentrations, as influencing pectase gel rmation. He found that for a 1 per cent pectin solution, the most rapid co-gulation was obtained with a concentration of 0.15 per cent calcium chloride pH 4.8-5.0. Kertesz (71) points out that these values hold only for pectin ncentration of 1 per cent. It is necessary, therefore, that studies of pectase l formation be carried out at definite pectin, calcium, and hydrogen ion ncentration. If these conditions are followed the data will correlate with her data similarly obtained.

Kopaczewski (72) has shown that the alkali earth metals cause the for-ation of pectase gels, and that Cu and Fe salts also exhibit similar prop-rties. In fact, the latter have a much greater action in this respect than oes Ca.

SUMMARY

Pectic enzymes occur in varying concentrations in plant materials and ause changes during growth and decay. The classification of these enzymes s difficult because of lack of exact knowledge of their substrates and end-oducts. Usually three different enzymes are recognized. These have been med protopectinase, pectinase, and pectase.

The pectic enzymes are of commercial importance in all processes involv-g plant materials. Some of these enzymatic effects are desirable; others are sirable. Ripening in storage, retting of flax, clarification of fruit juices wines are common desirable processes. Overripening and decay are the f harmful effects.

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Chapter VII

METHODS OF ANALYSIS FOR PECTIN

This chapter discusses used and proposed methods for pectin analysis. e present state of knowledge discloses that no method, reaction, or physical operty is strictly specific for pectin because other compounds associated th it in plant tissues are likewise responsive. For this reason, for example, rtain precipitation methods are partly or largely inaccurate. The interfer- compounds jointly precipitate with the pectin. Also with physical prop- ties, as for example, with viscosity, errors are introduced by the associated mpounds.

Methods based on quantitative estimation of degradation products of ctin, for examples of pectic acid, methyl alcohol, or furfural, do not give curate analysis of the pectin content, because the quantitative relation- ip of pectin with its derivatives is unknown. When the constitution of the ctin molecule has been established, perhaps absolute methods of analysis n be developed.

From the several methods hitherto used, it is possible to choose one that tisfies the requirements of a particular problem, even though the data of o different methods do not correlate. Usually only comparative data are ught when dealing continuously with one phase of study, hence either ese or rapid, practical, approximate methods are useful. The latter are npirical and may depend upon the judgment and skill of the operator.

The housewife finds that a pectin concentration high enough to "set" her lly has been reached when the boiling juice "sheets" off the spoon. Geret) mixes equal volumes of alcohol and fruit juice or pectin solution in a arrow tube and, after it has stood one hour at 0° C., estimates the pectin comparing its mass with standards prepared in the same manner from nown pectin solutions. Fellers and Rice (2) developed a rapid centrifuge ethod based on the same principle.

Other methods of pectin analysis involve hydrolysis and precipitations of ectic acid or its salts. Some methods based on hydrolysis involve estima- on of the methyl alcohol, carbon dioxide produced, or alkali consumed. lso there are methods based on measurements of viscosity or color. Most these methods will be given verbatim with some discussion of their limita- ons and errors.

The earliest analytical method comprised precipitation of the pectin by ddition of two volumes of alcohol, drying, and weighing as pectin. The ata thus obtained are always high, because of co-precipitation of other soluble material such as proteins, dextrins, gums, enzymes, microorgan- ms, salts, etc. Furthermore, Carré (3) demonstrated that pectin fails to recipitate from alcoholic solutions when present in concentrations less than 5 gms. per liter of the original solution.

Wichmann (4) devised a method of alcohol precipitation in which the occulation of small quantities of pectin in the alcohol is accomplished by the ddition of an electrolyte. In low concentrations, owing to their charged ondition, the pectin particles remain colloidally dispersed in the alcoholic edium even though the hydrophilic effect is reduced. Wichmann found hat small quantities of hydrochloric acid flocculated the pectin by neutraliz- ng the charge on the pectin micellæ. It has also been found (5) that a very mall concentration of calcium chloride in the alcohol yields even better esults.

The alcohol method of analysis worked out by Wichmann (6) and recommended by the Association of Official Agricultural Chemists is as follows:

"Evaporate 100 cc. of the sample solution to 20 cc. after adding 1-2 lumps of cube sugar, if sugar is not already present. (More sugar may have to be added if water insoluble substances separate during the evaporation.) Cool to room temperature. Add slowly and with constant stirring 200 cc. of 95 per cent alcohol. Allow to stand one hour or overnight. Filter on a smooth qualitative paper and wash the precipitate well with 95 per cent alcohol. Wash the precipitate back into the original beaker with a stream of hot water. Wash the paper well. Evaporate the solution to 20 cc. and add 5 cc. of 10 per cent hydrochloric acid. If water insoluble solids have separated, stir well and, if necessary, warm slightly to dissolve. If the quantity of the alcohol precipitate, as indicated by its volume in the first precipitation, is not excessive, add ½ gram of **acid-treated** and ignited asbestos. Again precipitate with 200 cc. of 95 per cent alcohol as before. Allow to stand one hour and filter into a Gooch crucible provided with a **thin** asbestos mat. Wash well with alcohol of over 85 per cent strength, suck dry, and dry in a water oven. Weigh, ignite, and weigh again. The loss in weight is alcohol precipitate. If the quantity of the alcohol precipitate in the first precipitation appears to be large, it is better to filter the second time on paper to avoid the clogging of the Gooch. Wash the precipitate well with the wash alcohol to remove all the hydrochloric acid. Then wash the precipitate into a platinum dish for drying and ignition. Exercise care so that no loss of precipitate occurs at any point, as in many cases it may be so nearly colorless as to be almost invisible."

If the amount of alcohol precipitate is great, the Gooch crucible may clog and the filtration will stop. In such a case it is advisable to filter through paper, transfer completely to a dish, and then filter through the Gooch. To transfer the precipitate completely, remove with a spatula as much as possible without including fibers of the filter paper, dissolve the residual adhering pectin through the filter paper with several small portions of water, combine the transferred precipitate and filtrate and add three volumes of alcohol. If too much water was not used, the precipitate now can be filtered through the Gooch crucible.

Precipitation as Pectic Acid or Pectates

Wichmann and Chernoff (7) convert pectin into pectic acid, weigh the precipitate, and thus estimate the pectin concentration.

"Add 2-4 lumps of cube sugar to 200 cc. of sample solution, if this does not already contain sugar, and evaporate to 25 cc. Precipitate with 200 cc. of 95 per cent alcohol, allow to settle, filter, and wash with 95 per cent alcohol. Transfer the precipitate into the original beaker with hot water. Evaporate the pectin solution to 40 cc. and cool to 25° C., or below. If the water insoluble substances should separate during evaporation, stir vigorously and, if necessary, add a few drops of 10 per cent hydrochloric acid and warm. Then cool again and add 2-5 cc. of 10 per cent sodium hydroxide (the bulk of the precipitate will indicate approximately the quantity to use) in sufficient water to make a total volume of 50 cc. Allow to stand 15 minutes, add 40 cc. of water and 10 cc. of 10 per cent hydrochloric acid, and boil 5 minutes. Filter the pectic acid on a qualitative filter paper and wash with hot water. The filtration should be rapid and the filtrate clear. If this is cloudy or of a colloidal nature, the results should be rejected and

the determination repeated with the addition of more alkali. Wash the pectic acid back into the beaker, adjust to 40 cc., cool to below 25° C., and repeat the saponification and precipitation just as described. Filter and wash the pectic acid with hot water only to the point where a test of the filtrate shows a negligible quantity of acid. More than 500 cc. of total filtrate should not be necessary. Then wash the pectic acid into a platinum dish and dry on a steam bath and finally in a water oven to constant weight. Weigh, ignite, and weigh again. The loss in weight is pectic acid."

The weighed product is considered to be di-galacturonic acid and differs from the pectic acid of Carré's Method, which yields tetra-galacturonic acid. Besides yielding a product of uncertain relationship to the original pectin, this method is objectionable because decomposition of the uronic acids is indicated in the formation of furfural during the treatment. It is possible, however, that the furfural is formed from pentoses originally present and not from the uronic acids. Due to this uncertainty, and the fact that the uronic acids easily decompose to furfural, question must exist as to the reliability of this method.

Pectic acid is soluble in pure water and precipitates only in the presence of electrolytes. Thus low results may be obtained by prolonged washing with distilled water. It is better to discontinue the washings while they still appear faintly acid to litmus. During alkaline hydrolysis the temperature must be controlled carefully, because heating over 30° C. causes serious losses.

Calcium Pectate

The pectic acid of Carré and Haynes (8), which results from mild alkaline hydrolysis of pectin, is weighed as calcium pectate and this is claimed to be a definite equivalent of pectin. However, owing to the existence of a large number of pectins, it is questionable whether any chemical equivalent can be assumed.

"A quantity of pectin is taken which will yield from 0.02-0.03 gram of calcium pectate; this is neutralized and then diluted to a volume such that after addition of all reagents the total volume measures about 500 cc.—100 cc. of N/10 NaOH are then added and the mixture is allowed to stand at least an hour, but preferably overnight. Fifty cc. of N/1 acetic acid are then added, and after five minutes 50 cc. of M/1 $CaCl_2$. The mixture is then allowed to stand for an hour, after which it is boiled for a few minutes and filtered through a large fluted filter. If the precipitation has been properly carried out, filtration should take place very rapidly and subsequent washing should be easy. The washing is continued with boiling water until the filtrate is free from chloride, after which the precipitate is washed back into the beaker, boiled, and filtered again. It is then tested for chloride, and these processes are repeated until the filtrate from the boiled precipitate gives no indication of chloride with silver nitrate. It is then filtered into a small fluted filter, from which it can be transferred to a dish and finally to a Gooch crucible which has been previously dried to 100° C. The precipitate is dried to a constant weight at 100° C., which has been found to require about twelve hours."

More consistent data can be obtained if a preliminary precipitation of the pectin is made with acidified alcohol. The filtered precipitate is redissolved and treated in the manner described. This partially eliminates absorption of extraneous materials by the calcium pectate precipitate.

Objections to the Carré method reside in the facts that it is not a direct measure of pectin and that the precipitate is colloidal and is difficult to filter and wash. Sometimes as many as fifty washings are necessary to free the precipitate of adsorbed ions. There is also some doubt as to whether the composition of the calcium compound is constant (9), although the authors state that the calcium content of 7.66 per cent, calculated from the formula $C_{17}H_{22}O_{18}$ Ca., is in very close agreement with that found by analysing the calcium pectate precipitate. Subsequent work by other authors shows agreement between the theoretical and experimental values for the calcium content. This method has been, and still is, in general use even though it is subject to the limitations stated above.

Viscometric Methods

The possible utilization of viscosity measurements as a measurement of pectin concentration in studying gelation was early pointed out by Weltzien-muth (10) in her work on conditions influencing pectin gel formation.

Further consideration of this relationship between the viscosity and jellying power of pectin solutions was embodied in a comprehensive investigation carried out by Myers and Baker (11). They found that the viscosity varied directly as the sugar-carrying capacity of the pectin solution. However, in a later publication (12), they state that the method is of only limited application, but may be used to determine the jelly grade of pectins prepared under the same conditions of extraction.

Wilson (13) confirms Myers' and Baker's findings and states that the method had been used in their laboratories for three years to grade commercial pectin preparations. Wilson's method follows:

"Measure 94 cc. distilled water. Weigh out 0.25 grams of tartaric acid—Na_2CO_3 mixture, 5 grams of dry, fine granulated sugar, and one gram of pectin, using an aluminum scoop, and transfer all ingredients to a perfectly dry four-ounce narrow-necked bottle (grape juice type). Mix thoroughly in the bottle while dry. Add about 50 cc. of distilled water, stopper with a rubber stopper or the thumb, and shake vigorously for a few seconds. Remove the stopper or thumb cautiously and allow CO_2 to escape without loss of any of the solution. Add the remainder of the 94 cc. of water, thus making a 1 per cent pectin dispersion with a sufficient degree of accuracy. Shake for five to ten minutes, or until the last visible particle of pectin has disappeared. If there are any lumps of undispersed material the sample should be discarded and a new one prepared. A very small amount of undissolved pectin will cause a large error. After dispersion is complete let the solution stand for a few minutes, until most of the gas bubbles have disappeared, pour about 50 cc. into a small beaker, adjust the temperature to exactly 25° C. (or to the temperature at which the pipette being used is standardized), and take the time of flow through the pipette, using the stop watch. Take the average of several readings, which should check within a few tenths of a second, as to the time of flow. By referring to a standard curve of jelly grade as time of flow the jelly grade may be read off directly."

Because the viscosity is a function of other conditions such as pH, presence of heavy salts, the relative proportions of pectic substances present, etc., Wilson concludes that this method is not absolutely reliable although it does possess practical value. Enough exceptions to the rule were noted by Wilson to prevent acceptance of the viscosity method as a final assay for pectin.

Nevertheless, in following changes in pectin concentration (14), and in determining the jelly power of pectin extracts (15), viscosity methods are being used increasingly. Baker (16) discusses a simple viscosity method for rating fruit juices for jelly making. He holds that solutions with relative viscosities below four require the addition of pectin; those above twelve require dilution with water or fruit juices.

In collaboration with P. B. Myers (17), the same author explains the relationship of viscosity to jellying power. Assuming that jellying power is directly dependent upon polymerization of the pectin molecule, he holds that any treatment causing decomposition of the pectin molecule, but not causing depolymerization, decreases the viscosity but not necessarily the jellying power. Since depolymerization does reduce the jellying power, it is permissible to correlate this property with viscosity.

Saponification

In 1927 (18) a committee formed for the purpose of considering the various methods proposed for pectin analysis concluded that because of its ease of operation the saponification method of Ahmann (19) was especially to be recommended. Accompanying this recommendation, it is pointed out that no absolutely satisfactory method for estimating pectin had yet been presented and that further study is highly desirable. Ahmann's method is as follows:

"To pectin solutions containing from 0.25–1.0 gm. in 200 cc. of solution a known amount of alkali (50 cc.) was added from a pipette so that the concentration of the alkali would be about 0.1 normal. The solutions were then made up to volume (250 cc.) and allowed to stand at 55° C. for twelve hours. The flasks were sealed during hydrolysis in order to prevent the entrance of carbon dioxide. Aliquots were then pipetted off and titrated with hydrochloric acid. The sodium hydroxide should be about four times as strong as the hydrochloric acid for the back titration in order that the difference will be large and so reduce the percentage of error. From the number of cubic centimeters of alkali combined with the pectin the amount of pectin is calculated. Taking the neutral equivalent of pectic acid at 55° C. as 194.9, which is equivalent to 208.9 grams of pectin, the following method can be used to calculate the amount of pectin from the amount of alkali used:

$$\frac{\text{NaOH}}{\text{Pectin}} = \frac{\text{weight of alkali combined}}{X}$$

$$\frac{40}{208.9} =$$

The following precautions must be taken to insure comparable results. In case very small quantities of pectin are found (0.010 grams in 50 cc. of solution) about a 200 cc. sample should be taken. As the alkali is four times as strong as the acid, care must be taken in pipetting the alkali for saponification since a drop more in one than in another makes an error of about 0.2 of a cc."

Notwithstanding the committee's recommendation, this method has not received general approval, as is evidenced by preference shown for other methods in most subsequent publications.

Determination of Pectin by Methoxyl Content

Von Fellenberg (20) proposed a method of analysis based on his discovery that methoxyl groups occur in all pectins. He assumed that the number of methoxyl groups in pectin is constant. It has since been found that the per

cent of methoxyl varies with the source, treatment, etc., so that this method may be erroneous and has little value for quantitative analysis.

The methoxyl content does provide a quantitative method for differentiating pectins from some of the related polysaccharides. It also measures the degree of hydrolysis of the fully esterified pectin to form pectic acid. It may indicate the jelly power of pectin because good jellying pectins usually contain from 7-12 per cent of methoxyl.

Evolution of Carbon Dioxide

When pectin is boiled with 12 per cent hydrochloric acid it decomposes and yields carbon dioxide; this carbon dioxide shows some relationship to the original pectin. However, because there exists a variety of pectins, this method, like the methoxyl method, must be considered a qualitative rather than quantitative measure of the pectin content.

Colorimetric Measurements

Silin and Silina (22) recommend the following method based on estimation of furfural from pectin.

"Distil a mixture of 5 cc. of the sample, 2.5 cc. of concentrated HCl and 32.5 cc. of HCl (sp. gr. 1.06), in such a manner that 30 cc. are collected in three hours. The final distillate is made neutral to phenolphthalein with 10 per cent sodium hydroxide and 4 cc. of acetic acid and 0.5 cc. of aniline are added. This solution is compared in a Dubosq colorimeter with a standard prepared by treating a sample of furfural stock solution in a similar manner. The conversion factors to pectic acid and pectin are 3.8 and 5.5, respectively."

Evidently this method is not reliable because the furfural liberated from the different pectins is not constant and pentoses also occur in plants.

Extraction of Pectin for Analysis

Because pectin may be present in any one or all of its several forms—protopectin, pectin, pectinic acids, pectic acid, and pectates—it is obvious that each of these requires a different technique of extraction. Extraction with boiling water or with dilute acids is unsatisfactory in estimating the total pectic content because only the soluble pectin, pectinic acids, and part of the protopectin dissolve. Continued boiling to dissolve the protopectin is objectionable because of accompanying hydrolysis of the pectin already in solution. By employing several successive short extractions, this latter effect can be minimized so that ultimately all of the protopectin is dissolved without substantial decomposition. Extraction with dilute alkali readily effects the solution of the entire group, but since this reagent decomposes the pectin to products of indefinite composition, there is no basis for estimating the form and concentration of the original pectin.

For complete analysis of pectic compositions Nanji and Norman (23) have designed a system involving several steps, employing different solvents for the several components. They extract successively with (1) water, (2) 0.5 per cent oxalic acid, and (3) 0.5 per cent ammonium oxalate. The pectic compound in each is then estimated by the Carré method and the data affords a basis of calculation successively of the free pectin, the pectin in combination with metallic ions and the pectic acid. This method is based on the solubility properties of these constituents: the water soluble pectin; water

insoluble but acid soluble pectates; water and acid insoluble but ammonia soluble pectic acid.

Most methods of pectin analysis are based on preliminary extractions with dilute acids quite similar to the commercial extraction of pectin from fruits. Making use of acid extraction, Stewart (24) designed a good quantitative method:

"Weigh 5 grams of pomace into a 250 cc. beaker, add 74 cc. of water and 1 c.c. of N/HCl. Boil for 30 minutes, keeping the volume approximately constant. Filter through a 9 cm. paper on a small Buchner funnel. Wash with boiling water, squeezing the pomace a little with a glass rod. Make the filtrate up to 100 cc., after cooling. With the help of the glass rod and boiling wash bottle transfer the pomace again to the beaker, add 5 cc. of N/HCl, and make up to the same volumes as before. Boil for 20 minutes and filter as before. Make up the second extract to 100 cc. For the third extraction use 25 cc. of N/HCl and 50 cc. water. Boil for 10 minutes. Filter again, still using the same paper, and make the filtrate and washings up to 100 cc. Then mix the three extracts. Take 10 cc. for the determination, add 80 cc. of industrial methylated spirit and 9 cc. of N/HCl, mix and stir. Leave for one hour, filter off the pectin, wash with 80 per cent acidified alcohol (N/10), and finally with strong alcohol. The pectin may be transferred to an evaporating basin with boiling water and then dried. Weight x 600= % pectin."

The time of heating is decreased and the acidity is increased for successive extractions to obtain the maximum yield of pectin.

Enzyme Action

A condition that is often overlooked in analysis of plant materials is the possibility of enzyme actions altering the nature of the substance sought. In pectin analysis the products of these actions are always such that they do not respond to the particular method used and therefore render such analysis worthless. A further common mistake is the belief that certain fruits are incapable of forming jellies. This is not always true. Fruits possessing highly active pectic enzymes, with certain treatment, lose jellying power before the enzyme is inactivated.

One method of preventing pectic enzyme action is to grind the material in alcohol and boiling. Cold alcohol treatment does not deactivate the enzyme but merely causes its reversible coagulation. The pulp is filtered off and the analysis is pursued in the usual manner. The inference that the pectin is not affected by alcohol must be accepted with reservation. There is some evidence to the contrary, such as the dissolution of the araban portion and alteration of the jellying properties.

A successful method (5) is to grind the material directly in the extracting solution. The acidity (pH= 2.0) is great enough to prevent enzyme action. This method is simple and inhibits enzymatic action.

Grinding the Plant Material

Obviously the efficiency of the extraction depends upon the fineness of the ground material. It is customary to put the fruit or vegetable through a food chopper several times. If very fine comminution is desired, the material can be ground in a mortar with sand.

More rapid extraction is obtained by stirring the pulp vigorously while heating. However, this procedure, as well as the practice of grinding the

material very fine, leads to difficulty in filtration. In some cases the despersion becomes so complete that it is impossible to clarify the extract, even in a high speed centrifuge.

Miscellaneous

When plant materials, and especially extracts, are allowed to stand for some time, mold and yeast act to destroy the pectin. It is good practice either to proceed directly with the analysis or preserve the material or the extracts by (1) pasteurizing, (2) toluene or chloroform, (3) or in a refrigerator at a temperature slightly above freezing.

If the hot extract is very large in volume, some hydrolysis of the pectin takes place before it cools. This effect can be minimized by rapid cooling.

The optimum pH for extraction is rather definite at 2.0-2.2. It is not sufficient, however, simply to use an extraction solution of this pH because certain plants contain buffering materials that change this. For precise work it is best to check the adjustment of the pH electrometrically. In continuous studies on the same plant materials it is advantageous to determine the limits of the buffering effect and include this in the calculation of the acid requirement.

Sometimes it is desirable to determine, either qualitatively or quantitatively, some of the hydrolytic products of pectin. The following section is devoted to methods that are in general use for this purpose.

Methoxyl Determination

Zeisel's (25) method is carried out in the following manner:

"The material (0.3 gm.) is placed in a distilling flask (130 cc.) having its side arm connected with a carbon dioxide generator. A delivery tube runs vertically for fifty centimeters and then bends back down into two U-tubes connected in series. The first of these contains distilled water and a few milligrams of red phosphorus. The second contains only distilled water. These tubes are so arranged that they can be placed in a water bath. From the second U-tube a delivery tube runs to the absorption apparatus, which consists of an Erlemeyer flask and a Fresenius nitrogen bulb.

In the first and second absorption flasks are placed 35 and 15 cc. respectively of freshly alcoholic silver nitrate solution. The silver nitrate solution is composed of 10 grams silver nitrate dissolved in 25 cc. water and 225 cc. of 95 per cent alcohol. To the decomposition flask is added 15 cc. of hydroiodic acid (d.-1.70). The mixture is heated to 130° C. in a paraffin bath and maintained at 130-140° C., while a slow stream of carbon dioxide is passed through the apparatus. The water bath surrounding the U-tubes is kept at 50°-60° C. As the operation proceeds, the silver iodide precipitate settles out, leaving a clear supernatant liquid.

This precipitate is washed with sufficient water to make a volume of 500 cc., the solution is evaporated to 150-200 cc. to remove alcohol, and a few drops of nitric acid are added. The solution is then made up to 500 cc., digested on a steam bath one-half hour, filtered through a Gooch crucible, washed, dried at 120° C. for two hours, and weighed. Weight x 0.132= Methoxyl."

Von Fellenberg (26) worked out a colorimetric analysis involving hydrolysis by NaOH, and determining in a Dubosq colorimeter, the methanol formed. Methlitz (27) reports agreement between this method and that of

Zeisel within 3.5 per cent when the Dubosq colorimeter is used. When using color standards, he reports errors of 5.5 per cent.

A rapid method (28, 29) for methoxyl determination is described by Romeo:

"Dissolve 1.0 gm. of pectin in 250 cc. H_2O; heat to hasten solution. Replace the H_2O lost by evaporation, cool to 20° C., neutralize by 0.5 N/NaOH, with phenolphthalein as indicator. Add 20 cc. 0.5 N/NaOH, stir, allow to stand 15 minutes. Add 20 cc. 0.5 N/H_2SO_4 and titrate excess acid with NaOH; 1 cc. of 0.5 NaOH corresponds to 0.0155 gm. methoxyl."

This method compares very favorably with that of Zeisel.

A micro method is described by Nanji and Norman (30) in which the material is treated overnight in a closed vessel with 0.05 N/NaOH, and H_2SO_4 added until the pH= 5.0. The mixture is distilled and the alcohol is determined in the distillate.

Pentose Estimation—(31)(32)(33)(34)

QUALITATIVE ANALYSIS

d-Galactose:

This sugar is fermented by brewer's yeast at pH= 5.5. It can be identified as mucic acid. Ortho-tolyl hydrazine (35) is also a test for galactose. One part of the sugar in an equal amount of water is heated for 30 minutes with one part of ortho-tolyl hydrazine, dissolved in twenty parts of absolute alcohol. After 24 hours the hydrazone is filtered off, washed with alcohol and ether and recrystallized from 95 per cent alcohol. M. P. 176°.

l-Arabinose

Arabinose is not fermented by yeast. Its benzyl hydrazone (m. p. 174°) and p-bromo-phenylhydrazone (m. p. 161°-163°) are characteristic. They are prepared by treating a 1 per cent solution of the pectin with hydrazine solution consisting of one part of the hydrazine, 3.5 parts of 50% acetic acid and twelve parts of water. Two parts of the hydrazine are used for one part of arabinose.

d-Galacturonic Acid

Upon treating aqueous solutions of d-galacturonic acid with a little freshly filtered basic lead acetate solution, a colorless flocculent precipitate is formed (36). As more reagent is added, this redissolves and can be reprecipitated on heating as a blood- to brick-red precipitate.

Glucuronic acid does not give the red precipitate on further heating. Gluconic, galacturonic and arabonic acids do not give any precipitate on heating. Saccharic and mucic acids give white precipitates that do not change on heating. Pyruvic, hydroxypruvic, saccharaic, and hydroxymaleic all give reactions other than that for galacturonic. It is possible to detect as little as 5 mg. per liter of the acid in this way. If allowed to stand several hours, solutions containing as little as 1 mg. per liter show faint red precipitates.

SUMMARY OF ANALYTICAL METHODS

No analytical method gives an exact measurement of the pectin content of plant materials. Comparative results can be obtained by the use of a particular method. However, data obtained by one method can not be correlated exactly with data obtained by other methods.

The calcium pectate method is probably the most accurate method of

pectin analysis, although materials are found in plants that precipitate under the same conditions. The alcohol method would be the best, if it were not for the co-precipitation of many other plant materials with the pectin. For analysis of pectin solutions free from interfering materials, the alcohol method is time-saving and involves a minimum of alteration of the pectin molecule.

The method of measuring the specific viscosity of a pectin-acid-sugar solution and comparing this with a standard curve of viscosity vs. grams of pectin is a measurement of pectin concentration. This method is based on the only specific property of pectin known, namely, the formation of sugar-acid gels. The presence of certain foreign substances interfere with gel formation, which reduces the application of the method.

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